

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 31/195	A1	(11) International Publication Number: WO 99/62509 (43) International Publication Date: 9 December 1999 (09.12.99)
(21) International Application Number: PCT/US99/11876 (22) International Filing Date: 1 June 1999 (01.06.99) (30) Priority Data: 60/087,556 1 June 1998 (01.06.98) US 09/321,584 28 May 1999 (28.05.99) US (71) Applicant (for all designated States except US): NITROMED, INC. [US/US]; 12 Oak Park Drive, Bedford, MA 01730 (US). (72) Inventor; and (75) Inventor/Applicant (for US only): SAENZ DE TEJADA, Inigo [US/ES]; San Rafael, 14, Posuelo de Alarcon, E-28224 Madrid (ES). (74) Agents: GRIEFF, Edward, D.; Hale and Dorr LLP, 1455 Pennsylvania Avenue, N.W., Washington, DC 20004 (US) et al.		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, HU, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: ENDOGENOUS NITRIC OXIDE SYNTHESIS UNDER CONDITIONS OF LOW OXYGEN TENSION (57) Abstract <p>The present invention provides methods of promoting synthesis of nitric oxide or endothelium-derived relaxing factor (EDRF) in hypoxic mammalian tissues by administering at least one N-hydroxyguanidine compound that is a substrate of nitric oxide synthase, and, optionally, one or more vasoactive agents. The present invention also provides methods of promoting relaxation of vascular and non-vascular smooth muscle and treating sexual dysfunctions in patients by administering at least one N-hydroxyguanidine compound that is a substrate for nitric oxide synthase, and, optionally, one or more vasoactive agents. The present invention also provides methods for treating clinical conditions resulting from hypoxic conditions, such as pulmonary disease, cardiovascular disorders, circulatory hypoxia, specific organ hypoxia, localized hypoxia, edema, central nervous system disorders, memory loss, or arterial disease. The present invention also provides methods for treating clinical conditions associated with deficient nitric oxide pathways by administering at least one N-hydroxyguanidine compound and, optionally, one or more vasoactive agents. The present invention also provides pharmaceutical compositions comprising at least one N-hydroxyguanidine compound and, optionally, one or more vasoactive agents.</p>		

BEST AVAILABLE COPY

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

ENDOGENOUS NITRIC OXIDE SYNTHESIS UNDER CONDITIONS OF LOW OXYGEN TENSION

This application claims priority to U.S. Provisional Application No. 60/087,556, filed June 1, 1998.

5

FIELD OF THE INVENTION

The present invention describes novel methods to induce synthesis of endogenous nitric oxide or endothelium-derived relaxing factor, and methods for maintaining levels of nitric oxide under hypoxic conditions. One aspect of the invention relates to novel methods to induce vasodilation. The present invention
10 also provides methods for treating or preventing sexual dysfunctions in males and females by administering at least one N-hydroxyguanidine compound, such as N-hydroxy-L-arginine, and, optionally, one or more vasoactive agents. The present invention also provides methods for treating clinical conditions resulting from hypoxic conditions, such as, pulmonary disease, cardiovascular disorders,
15 circulatory hypoxia, specific organ hypoxia, localized hypoxia, edema, central nervous system disorders, memory loss, or arterial disease. The present invention also provides methods for treating clinical conditions associated with deficient nitric oxide pathways by administering at least one N-hydroxyguanidine compound, and, optionally, one or more vasoactive agents. The present invention
20 also provides novel compositions comprising at least one N-hydroxyguanidine compound, and, optionally, one or more vasoactive agents. The N-hydroxyguanidine compounds in the present invention are substrates for nitric oxide synthase.

BACKGROUND OF THE INVENTION

25 Nitric oxide is a small diatomic molecule with multiple biological actions, including inhibition of platelet adhesion and aggregation, and relaxation of vascular and non-vascular smooth muscles. Nitric oxide has also been reported to have anti-inflammatory, anti-bacterial and anti-viral properties (see, review by Moncada et al., *Pharmacol. Rev.*, 43:109-142 (1991)). In the gaseous state, nitric
30 oxide exists as a lipophilic molecule in a neutral redox state (NO). Nitric oxide is a complex molecule since it is able to exist in multiple redox states under different physiological conditions. It can also formally exist in charged forms i.e., nitrosonium (NO⁺) or nitroxyl (NO⁻), or as the neutral species, nitric oxide (NO·).

In biological tissues, nitric oxide has a very short half-life, estimated at less than one second.

One of the potent actions of nitric oxide in mammals is to relax vascular and non-vascular tissue, and, as such, either nitric oxide or an adduct that delivers
5 nitric oxide, is useful as a vasodilator. In the mammalian body, endogenous nitric oxide is produced through an enzymatic reaction in which nitric oxide synthases use L-arginine and molecular oxygen for the synthesis of nitric oxide and citrulline. One of the actions of nitric oxide is believed to be the activation of a soluble form of guanylate cyclase, a cellular enzyme, which catalyzes the
10 formation of 3',5'-cyclic guanosine monophosphate (cGMP). cGMP is believed to act on other cellular targets to mediate the relaxation of vascular smooth muscle and provide the therapeutic effect of vasodilation. Another action of nitric oxide is believed to be the regulation of $\text{Na}^{(+)}\text{-K}^{(+)}\text{-ATPase}$.

The synthesis of nitric oxide from L-arginine by nitric oxide synthase occurs
15 in two steps, each of which requires NADPH. In the first step, an intermediate N-hydroxyguanidine product, N^{G} -hydroxy-L-arginine, is synthesized by the incorporation of an oxygen into the guanidine function of the L-arginine molecule. In the second step, a second oxygen is incorporated into N^{G} -hydroxy-L-arginine to form L-citrulline and nitric oxide. (Fukuto et al, in *Methods in Nitric Oxide*
20 *Research*, Feelisch et al, eds., John Wiley & Sons, Ltd., pp. 147-160 (1996)). Under an environment of low oxygen tension, however, the synthesis of nitric oxide is greatly reduced. (Furchgott et al, *Nature*, 288(5789):373-376 (1980); Johns et al, *Circ. Res.*, 65(6):1508-1515 (1989)).

Several clinical conditions are associated with low oxygen tension, such as
25 sexual dysfunctions (Kim et al, *J. Clin. Invest.* 91(2):437-442 (1993)), pulmonary diseases (including respiratory distress syndrome, asthma, bronchitis/emphysema, and chronic obstructive pulmonary disease) (Howes et al, *Thorax*, 51(5):516-519, (1996); Fagan et al, *Biochem. Biophys. Res. Commun.*, 254(1):100-103 (1999)),
circulatory hypoxia (including heart failure, strokes, and shock), specific organ
30 hypoxia (in which decreased circulation to a specific organ resulting in localized circulatory hypoxia can be due to organic arterial obstruction or can result as a consequence of vasoconstriction, e.g., Reynauds Syndrome) (Agusti et al, *Eur.*

Respir. J., 10(9):1962-1966 (1997)), localized hypoxia (which can result from venous obstruction and resultant congestions and reduced arterial blood inflow), edema (which increases the distance through which oxygen diffuses before it reaches the cells can also cause localized hypoxia), central nervous system disorders, memory loss, and arterial disease (Weitzberg et al, *Acta. Physiol. Scand.*, 143(4):451-452 (1991)).

Respiratory distress syndrome, in a child or adult, has severe consequences in the vasculature, such as pulmonary hypertension. Arterial insufficiency of the blood vessel of the penis leads to hypoxic ischemia of this tissue, which limits the synthesis of nitric oxide, and, therefore, limits the erectile capacity.

Increased oxygen requirements can also lead to low oxygen tension. For example, if the oxygen consumption of a tissue is elevated without a corresponding increase in volume flow per unit time, then the oxygen tension (P_{aO_2}) in the venous blood can be reduced. This can also occur when the hemoglobin is qualitatively and quantitative normal. Examples of such situations include fever and thyrotoxicosis in which cardiac output cannot rise normally, and also in cases in which metabolic rates of oxygen consumption are high.

It would be desirable to increase the production of nitric oxide in tissues under low oxygen conditions to activate the chain of biochemical and cellular events that lead to vasodilation. The present invention is directed to these, as well as other, important ends.

SUMMARY OF THE INVENTION

It has been discovered that administering one or more N-hydroxyguanidine compounds that are substrates for nitric oxide synthase, such as N-hydroxy-L-arginine, to tissues under conditions of low oxygen tension (hypoxia), results in the synthesis of nitric oxide, which is more effective than arginine in promoting the formation of cGMP and the relaxation of vascular and non-vascular smooth muscles.

One embodiment of the invention provides methods of promoting synthesis of nitric oxide in vascular and non-vascular cells of a mammal under low oxygen conditions comprising administering to the mammal a therapeutically effective amount of at least one N-hydroxyguanidine compound, such as N-hydroxy-L-

arginine, and, optionally, at least one vasoactive agent.

Another embodiment of the invention provides methods of promoting relaxation of vascular and non-vascular smooth muscle in mammalian tissue under low oxygen conditions comprising administering to the patient a
5 therapeutically effective amount of at least one N-hydroxyguanidine compound, such as N-hydroxy-L-arginine, and, optionally, at least one vasoactive agent.

Another embodiment of the invention provides methods of treating sexual dysfunctions in patients, including males and females, comprising administering to the patient a therapeutically effective amount of at least one N-
10 hydroxyguanidine compound, such as N-hydroxy-L-arginine, and, optionally, at least one vasoactive agent. Generally, the sexual dysfunctions are attributable to low oxygen conditions. Preferably, the sexual dysfunctions are attributable to hypoxic ischemia, neuropathy or arterial disease.

Another embodiment of the invention provides methods of promoting
15 synthesis of nitric oxide or endothelium-derived relaxing factor (EDRF) in hypoxic mammalian cells (low oxygen conditions) comprising administering to the patient a therapeutically effective amount of at least one N-hydroxyguanidine compound, such as N-hydroxy-L-arginine, and, optionally, at least one vasoactive agent.

Another embodiment of the invention provides methods to treat clinical
20 conditions associated with low oxygen tension, such as, pulmonary diseases, circulatory hypoxia, specific organ hypoxia, localized hypoxia, edema, central nervous system disorders, memory loss, or arterial disease, comprising administering to a patient in need thereof a therapeutically effective amount of at least one N-hydroxyguanidine compound, such as N-hydroxy-L-arginine, and,
25 optionally, at least one vasoactive agent.

Another embodiment of the invention provides methods to promote the synthesis of nitric oxide or endothelium-derived relaxing factor in mammals with deficient nitric oxide pathways comprising administering to the patient a therapeutically effective amount of at least one N-hydroxyguanidine compound,
30 such as N-hydroxy-L-arginine, and, optionally, at least one vasoactive agent.

Another embodiment of the invention provides pharmaceutical compositions comprising at least one N-hydroxyguanidine compound, such as N-

hydroxy-L-arginine, and a pharmaceutically acceptable carrier, and, optionally, at least one vasoactive agent. The compositions can also comprise an analog of N-hydroxy-L-arginine, and/or other active compounds.

5 These and other aspects of the present invention are described in more detail herein.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 is a measurement of cyclic GMP (cGMP) levels in rabbit corporal cavernosal tissues expressed as picomole of cGMP per milligram of protein. The prepared tissues were incubated with (a) vehicle control (filled bars) where a total of 10 samples were tested (n=10); (b) 300 μ M L-arginine (hatched bars) where a total of 19 samples were tested (n=19); (c) 30 μ M N-hydroxy-L-arginine (open bars) where a total of 20 samples were tested (n=20); and (d) 300 μ M N-hydroxy-L-arginine (stippled bars) where a total of 10 samples were tested.

Fig. 2A compares the effects of L-arginine and N-hydroxy-L-arginine on cGMP levels (expressed as cGMP per milligram of protein) in rabbit cavernosal tissues stimulated with 30 μ M acetylcholine (ACh) in the presence of 0% O₂. Tissues were incubated in physiological salt solution in the presence of 0% O₂ and were treated either with 30 μ M ACh or 300 μ M L-arginine or 300 μ M N-hydroxy-L-arginine in the presence of ACh. The cGMP levels for 10 samples of tissue were measured for each condition tested (n=10). * P < 0.05 vs ACh alone using one way ANOVA analysis followed by Student-Newmann-Keuls post-hoc test.

Fig. 2B shows the effects of L-arginine and N-hydroxy-L-arginine on cGMP levels in rabbit cavernosal tissues stimulated with 30 μ M acetylcholine (ACh) in the presence of 20% O₂. Tissues were incubated in physiological salt solution and bubbled with 20% O₂ and were treated either with 30 μ M ACh or 300 μ M L-arginine or 300 μ M N-hydroxy-L-arginine in the presence of 30 μ M ACh. * P < 0.05 vs ACh alone using one way ANOVA analysis followed by Student-Newmann-Keuls post-hoc test.

Fig. 3 shows that the inhibition of P450 metabolism by administering miconazole does not inhibit N-hydroxy-L-arginine induced relaxation of isolated rabbit cavernosal tissues. Tissues were incubated in physiological salt solution and bubbled with 20% O₂ and were treated with increasing concentrations of N-

hydroxy-L-arginine in the presence of a vehicle alone (Control, open circles) where a total of 6 samples were tested (n=6); 0.1 mM miconazole (closed circles) where a total of 3 samples were tested (n=3); or 0.1 mM miconazole plus 0.1 mM N^G-nitro-L-arginine (L-NNA, an inhibitor of nitric oxide synthase) (closed triangles) where
5 a total of 3 samples were tested (n=3). In the x-axis, log M [N-hydroxy-L-arginine] corresponds to ten fold increases of N-hydroxy-L-arginine from 1 μ M (at -6) to 1000 μ M (at -3). Data are expressed as mean \pm standard mean error of the percentage of total relaxation induced by 0.1 mM papaverine. * P < 0.01 by AVONA analysis.

10 Fig. 4A shows that two different inhibitors of corporal tissue relaxation, L-NNA (N^G-nitro-L-arginine, a nitric oxide synthase inhibitor) and ODQ (1H-[1,2,4]-oxadiazolo[4,3,a]quinoxalin-1-one, an inhibitor of soluble guanylate cyclase), are effective to inhibit N-hydroxy-L-arginine induced relaxation of corpus cavernosal tissue under normoxia conditions. Tissues were incubated in physiological salt
15 solution and bubbled with 20% O₂ and were treated with increasing concentrations of N-hydroxy-L-arginine in the presence of a vehicle alone (Control, open circles), 0.1 mM L-NNA (closed circles) or 0.02 mM ODQ (closed triangles). In the x-axis, log M [N-hydroxy-L-arginine] corresponds to ten fold increases of N-hydroxy-L-arginine from 1 μ M (at -6) to 1000 μ M (at -3). The relaxation of four
20 samples of tissue were measured for each condition shown (n=4). Data are expressed as mean \pm standard mean error of the percentage of total relaxation induced by 0.1 mM papaverine.

* P < 0.01 by AVONA analysis.

Fig. 4B shows the effect of two different inhibitors of corporal tissue
25 relaxation, L-NNA (a nitric oxide synthase inhibitor) and 1H-[1,2,4]-oxadiazolo[4,3,a]quinoxalin-1-one (ODQ, an inhibitor of soluble guanylate cyclase), on the hydroxylamine induced relaxation of corpus cavernosal tissue under normoxia conditions. Only ODQ inhibits hydroxylamine induced relaxation of corpus cavernosal tissue under normoxia conditions. Tissues were incubated in
30 physiological salt solution and bubbled with 20% O₂ and were treated with increasing concentrations of hydroxylamine in the presence of a vehicle alone (Control, open circles) where a total of 4 samples were tested (n=4); 0.1 mM L-

NNA (closed circles) where a total of 4 samples were tested (n=4); or 0.02 mM ODQ (closed triangles) where a total of 3 samples were tested (n=3). In the x-axis, log M [hydroxylamine] corresponds to ten fold increases of N-hydroxy-L-arginine from 1 nM (at -9) to 1000 μ M (at -3). Data are expressed as mean \pm standard mean error of the percentage of total relaxation induced by 0.1 mM papaverine. * P < 0.01 by AVONA analysis.

Fig. 5A shows that N-hydroxy-L-arginine is more potent than L-arginine for inducing corpus cavernosum tissue relaxation under normoxia conditions. Tissues were incubated in physiological salt solution and bubbled with 20% O₂. Each sample was stimulated to relax by administering 0.3 μ M acetylcholine, and, after the tissue was allowed to recover, various concentrations of either L-arginine (open circles) where a total of 4 samples were tested (n=4); or N-hydroxy-L-arginine (closed circles) where a total of 7 samples were tested (n=7) were administered and the relaxation of the tissue was again measured. In the x-axis, log M [drugs] corresponds to ten fold increases of either L-arginine or N-hydroxy-L-arginine from 1 μ M (at -6) to 1000 μ M (at -3). Data are expressed as mean \pm standard mean error of the percentage of total relaxation induced by 0.1 mM papaverine. ** P < 0.01 by AVONA analysis.

Fig. 5B shows that N-hydroxy-L-arginine is more potent than L-arginine to induce corpus cavernosum tissue relaxation under hypoxic conditions. Tissues were incubated in physiological salt solution and bubbled with 5% O₂. Each sample was stimulated to relax by administering 0.3 μ M acetylcholine, and, after the tissue was allowed to recover, various concentrations of either L-arginine (open circles) or N-hydroxy-L-arginine (closed circles) were administered and the relaxation of the tissue was again measured. In the x-axis, log M [drugs] corresponds to ten fold increases of either L-arginine or N-hydroxy-L-arginine from 1 μ M (at -6) to 1000 μ M (at -3). The relaxation of two samples of tissue were measured for each condition shown (n=2). Data are expressed as mean \pm standard mean error of the percentage of total relaxation induced by 0.1 mM papaverine. * P < 0.05% by AVONA analysis.

Fig. 6 shows that N-hydroxy-L-arginine is effective to induce relaxation of corpus cavernosum tissue under various oxygen concentrations. Tissues were

incubated in physiological salt solution and bubbled with the indicated concentration of O₂. 0% oxygen, open bars, total samples tested = 2. 1% oxygen, hatched bars, total samples tested = 3. 2% oxygen, shaded bars, total samples tested = 4. 5% oxygen, densely hatched bars, total samples tested = 4. 20% oxygen, filled bars, total samples tested = 2. * P < 0.05 compared to 20% oxygen by one way ANOVA analysis followed by Student Newmann-Keuls post-hoc test.

Fig. 7A shows that under normoxia conditions N-hydroxy-L-arginine is more potent than L-arginine to induce relaxation of aortic segments obtained from non-diabetic rats. Tissues were incubated in physiological salt solution and bubbled with 95% O₂ and were treated with vehicle alone (open circles) where a total of 2 samples were tested (n=2); L-arginine (closed circles) where a total of 5 samples were tested (n=5); or N-hydroxy-L-arginine (closed triangles) where a total of 11 samples were tested (n=11). In the x-axis, log M [drugs] corresponds to ten fold increases of either L-arginine or N-hydroxy-L-arginine from 100 nM (at -7) to 100 μ M (at -4). Data are expressed as mean \pm standard mean error of the percentage of total relaxation induced by 10 nM norepinephrine. * P < 0.01 compared to L-arginine by ANOVA analysis.

Fig. 7B shows that under normoxia conditions N-hydroxy-L-arginine is more potent than L-arginine to induce relaxation of aortic segments obtained from diabetic rats. Tissues were incubated in physiological salt solution and bubbled with 95% O₂ and were treated with vehicle alone (open circles) where a total of 6 samples were tested (n=6); or L-arginine (closed circles) where a total of 7 samples were tested (n=7); or N-hydroxy-L-arginine (closed triangles) where a total of 8 samples were tested (n=8). In the x-axis, log M [drugs] corresponds to ten fold increases of either L-arginine or N-hydroxy-L-arginine from 100 nM (at -7) to 100 μ M (at -4). Data are expressed as mean \pm standard mean error of the percentage of total relaxation induced by 10 nM norepinephrine. \uparrow P < 0.05 compared to vehicle and * P < 0.01 compared to L-arginine by ANOVA analysis.

Fig. 8 shows that under normoxia conditions N-hydroxy-L-arginine is more effective in inducing the relaxation of aortic segments obtained from diabetic rats than aortic segments obtained from non-diabetic rats. Tissues were incubated in a physiological salt solution and bubbled with 95% O₂ and were treated with

increasing concentrations of N-hydroxy-L-arginine. At lower concentrations of N-hydroxy-L-arginine (1 μ M), the tissue from diabetic rats (closed circles, total samples tested n=8) showed more relaxation than the tissue from non-diabetic rats (open circles, total samples tested n=11). In the x-axis, log M [N-hydroxy-L-arginine] corresponds to ten fold increases of N-hydroxy-L-arginine from 100 nM (at -7) to 100 μ M (at -4). Data are expressed as mean \pm standard mean error of the percentage of total relaxation induced by 10 nM norepinephrine. * P < 0.05 by one way ANOVA analysis followed by Student Newmann-Keuls post-hoc test.

Fig. 9A shows that under normoxia conditions L-NAME (N^G -nitro-L-arginine methyl ester, a nitric oxide synthase inhibitor) is effective to inhibit N-hydroxy-L-arginine induced relaxation of aortic rings isolated from non-diabetic rats. Tissues were incubated in physiological salt solution and bubbled with 95% O₂ and were treated with increasing concentrations of N-hydroxy-L-arginine in the presence of vehicle alone (Control, open circles, total samples tested n=11) or 10 μ M L-NNA (closed circles, total samples tested, n=3). In the x-axis, log M [N-hydroxy-L-arginine] corresponds to ten fold increases of N-hydroxy-L-arginine from 100 nM (at -7) to 100 μ M (at -4). Data are expressed as mean \pm standard mean error of the percentage of total relaxation induced by 10 nM norepinephrine. * P < 0.01 by ANOVA analysis.

Fig. 9B shows that under normoxia conditions L-NAME (N^G -nitro-L-arginine methyl ester, a nitric oxide synthase inhibitor) is effective to inhibit N-hydroxy-L-arginine induced relaxation of aortic rings isolated from diabetic rats. Tissues were incubated in physiological salt solution and bubbled with 95% O₂ and were treated with increasing concentrations of N-hydroxy-L-arginine in the presence of vehicle alone (Control, open circles, total samples tested n=8) or 10 μ M L-NNA (closed circles, total samples tested, n=8). In the x-axis, log M [N-hydroxy-L-arginine] corresponds to ten fold increases of N-hydroxy-L-arginine from 100 nM (at -7) to 100 μ M (at -4). Data are expressed as mean \pm standard mean error of the percentage of total relaxation induced by 10 nM norepinephrine. # P < 0.01 compared to control by ANOVA analysis.

Fig. 10A shows that in the presence of increasing concentrations of ACh under normoxia conditions L-arginine and N-hydroxy-L-arginine do not effect the

relaxation of aortic rings isolated from non-diabetic rats. Tissues were incubated in physiological salt solution and bubbled with 20% O₂ and were treated with vehicle alone (Control, open circles, total samples tested n=6); 10 μ M L-arginine (closed circles, total number of samples tested n=3); or 10 μ M N-hydroxy-L-arginine (closed triangles, total number of samples tested n=3) in the presence of ACh. In the x-axis, log M [ACh] corresponds to ten fold increases of acetylcholine from 10 nM (at -8) to 10 μ M (at -5). Data are expressed as mean \pm standard mean error of the percentage of total relaxation induced by 0.1 mM norepinephrine.

Fig. 10B shows that under normoxia conditions N-hydroxy-L-arginine is more effective than L-arginine in the presence of increasing concentrations of ACh to induce the relaxation of aortic segments isolated from diabetic rats. Tissues were incubated in physiological salt solution and bubbled with 20% O₂ and were treated with vehicle alone (Control, open circles, total samples tested n=6); or 10 μ M L-arginine (closed circles, total number of samples tested n=2) or 10 μ M N-hydroxy-L-arginine (closed triangles, total number of samples tested n=6) in the presence of ACh. In the x-axis, log M [ACh] corresponds to ten fold increases of acetylcholine from 10 nM (at -8) to 10 μ M (at -5). Data are expressed as mean \pm standard mean error of the percentage of total relaxation induced by 0.1 mM norepinephrine. * P < 0.01 compared to control by AVONA analysis.

Fig. 11A shows that under normoxia conditions N-hydroxy-L-arginine is more potent than L-arginine to induce the relaxation of aortic rings isolated from diabetic rats. Tissues were incubated in physiological salt solution and bubbled with 95% O₂. Each sample was stimulated to relax by administering 0.3 μ M acetylcholine, and, after the tissue was allowed to recover, various concentrations of either L-arginine (open circles, total number of samples tested n=5); or N-hydroxy-L-arginine (closed circles, one sample tested n=1), were administered, and the relaxation of the tissue was again measured. In the x-axis, log M [drugs] corresponds to ten fold increases of either L-arginine or N-hydroxy-L-arginine from 100 nM (at -7) to 1000 μ M (at -3). Data are expressed as mean \pm standard mean error of the percentage of total relaxation induced by 0.1 mM papaverine.

Fig. 11B shows that under normoxia conditions N-hydroxy-L-arginine is more potent than L-arginine to induce the relaxation of aortic rings isolated from

diabetic rats. Tissues were incubated in physiological salt solution and bubbled with 20% O₂. Each sample was stimulated to relax by administering 0.3 μ M acetylcholine and, after the tissue was allowed to recover, various concentrations of either L-arginine (open circles, total number of samples tested n=5); or N-hydroxy-L-arginine (closed circles, one sample tested n=1), were administered and the relaxation of the tissue was again measured. In the x-axis, log M [drugs] corresponds to ten fold increases of either L-arginine or N-hydroxy-L-arginine from 100 nM (at -7) to 1000 μ M (at -3). Data are expressed as mean \pm standard mean error of the percentage of total relaxation induced by 0.1 mM papaverine.

10 Fig. 11C shows that under hypoxia conditions N-hydroxy-L-arginine is more potent than L-arginine to induce the relaxation of aortic rings isolated from diabetic rats. Tissues were incubated in physiological salt solution in the presence of 0% O₂. Each sample was stimulated to relax by administering 0.3 μ M acetylcholine and, after the tissue was allowed to recover, various concentrations of either L-arginine (open circles, total number of samples tested n=5); or N-hydroxy-L-arginine (closed circles, one sample tested n=1), were administered, and the relaxation of the tissue was again measured. In the x-axis, log M [drugs] corresponds to ten fold increases of either L-arginine or N-hydroxy-L-arginine from 100 nM (at -7) to 1000 μ M (at -3). Data are expressed as mean \pm standard mean error of the percentage of total relaxation induced by 0.1 mM papaverine.

20 Fig. 12A shows that L-arginine decreases the perfusion pressure of non-diabetic rats more than the perfusion pressure of diabetic rats. The vasoactive response of the bolus infusion of increasing concentrations of L-arginine (ranging from 0.1 mg/kg to 10 mg/kg) to the left hindlimb of autoperfused (at a constant rate of 3.3 ml/min/kg) non-diabetic (open circles, total number of rats perfused n=3) or diabetic rats (closed circles, total number of rats perfused n=3) was measured. Data are expressed as mean \pm standard mean error of the percentage of pressure reached after the infusion of norepinephrine at 2 μ g/min/kg. * P < 0.05 compared to non-diabetic rats by AVONA analysis.

30 Fig. 12B shows that in the presence of increasing concentrations of N-hydroxy-L-arginine the perfusion pressure of non-diabetic and diabetic rats was very similar. The vasoactive response of the bolus infusion of increasing

concentrations of N-hydroxy-L-arginine (ranging from 0.1 mg/kg to 10 mg/kg) to the left hindlimb of autoperfused (at a constant rate of 3.3 ml/min/kg) non-diabetic (open circles, total number of rats perfused n=2) and diabetic rats (closed circles, total number of rats perfused n=3) was measured. Data are expressed as mean \pm standard mean error of the percentage of previous pressure reached after the infusion of norepinephrine at 2 μ g/min/kg.

DETAILED DESCRIPTION OF THE INVENTION

As used throughout the disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings.

10 "N-hydroxyguanidine" refers to any N-hydroxylated guanidine compound that is a substrate for nitric oxide synthase.

"N-hydroxy-L-arginine" refers to N^G-hydroxy-L-arginine.

"Patient" refers to animals, preferably mammals, more preferably humans.

15 "Transurethral" or "intraurethral" refers to the delivery of a drug into the urethra, such that the drug contacts and passes through the wall of the urethra and enters into the blood stream.

"Transdermal" refers to the delivery of a drug by passage through the skin and into the blood stream.

20 "Transmucosal" refers to delivery of a drug by passage of the drug through the mucosal tissue and into the blood stream.

"Penetration enhancement" or "permeation enhancement" refers to an increase in the permeability of the skin or mucosal tissue to a selected pharmacologically active agent such that the rate at which the drug permeates through the skin or mucosal tissue is increased.

25 "Carriers" or "vehicles" refer to carrier materials suitable for drug administration and include any such material known in the art such as, for example, any liquid, gel, solvent, liquid diluent, solubilizer, or the like, which is non-toxic and which does not interact with other components or the composition in a deleterious manner.

30 "Nitric oxide adduct" or "NO adduct" refers to compounds and functional groups which, under physiological conditions, can donate, release and/or directly or indirectly transfer any of the three redox forms of nitrogen monoxide (NO⁺,

NO⁻, NO[•]), such that the biological activity of the nitrogen monoxide species is expressed at the intended site of action.

It has now been discovered that nitric oxide (NO) synthesis or endothelium-derived relaxing factor (EDRF) can be promoted in tissues under low oxygen conditions (i.e., conditions of hypoxia) by administering a therapeutically effective amount of at least one N-hydroxyguanidine compound that is a substrate for nitric oxide synthase. The N-hydroxyguanidine compound can be administered in a pharmaceutically acceptable carrier either alone or in combination with other active compounds. N-hydroxyguanidine compounds include, for example, N-aryl-N'-hydroxyguanidine (such as, for example, N-(4-chlorophenyl)-N'-hydroxyguanidine)); nitrosated and/or nitrosylated N-aryl-N'-hydroxyguanidine; N-hydroxy-L-arginine; and analogs of N-hydroxy-L-arginine.

Analogues of N-hydroxy-L-arginine include, for example, N^ω-hydroxy-homo-L-arginine; carboxylic esters of N-hydroxy-L-arginine (including, but not limited to, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl and benzoyl esters); N-α derivatives of N-hydroxy-L-arginine (including, but not limited to, methyl, ethyl, and benzoyl derivatives, such as, for example, N-α-methyl-N^G-hydroxy-L-arginine, N-α-benzoyl-N^G-hydroxy-L-arginine, N-α-benzoyl-N^G-hydroxy-L-arginine ethyl ester); N^G-hydroxy-agmatine; N^G-hydroxy-L-argininic acid; nitrosated and/or nitrosylated derivatives of N-hydroxy-L-arginine (such as, for example, nitrosated and/or nitrosylated N-hydroxy-L-arginine, nitrosated and/or nitrosylated N^ω-hydroxy-homo-L-arginine, nitrosated and/or nitrosylated carboxylic esters of N-hydroxy-L-arginine, nitrosated and/or nitrosylated N-α derivatives of N-hydroxy-L-arginine, nitrosated and/or nitrosylated N^G-hydroxy-agmatine, and nitrosated and/or nitrosylated N^G-hydroxy-L-argininic acid). Preferred analogs of N-hydroxy-L-arginine include, for example, nitrosated and/or nitrosylated derivatives of N-hydroxy-L-arginine, more preferably nitrosated and/or nitrosylated N-hydroxy-L-arginine. The stable N^G-hydroxy-L-arginine nitric oxide adduct has been characterized by Hecker et al, *Proc. Natl. Acad. Sci.*, 92:4671-4675 (1995), and has been shown to be pharmacologically active.

As described and exemplified herein, the administration of N-hydroxy-L-arginine was observed to increase basal and acetylcholine (ACh) stimulated

production of nitric oxide (NO), as determined by measurement of cGMP in rabbit corpus cavernosum tissue. It was also observed that N-hydroxy-L-arginine was more effective than L-arginine in increasing the production of NO and the attendant relaxation of corpus cavernosum tissue under hypoxic conditions. The effectiveness of N-hydroxy-L-arginine to promote corpus cavernosum tissue relaxation under hypoxic conditions demonstrates that N-hydroxy-L-arginine will be useful to treat clinical conditions associated with vasoconstriction due to low oxygen tension or the need to elevate endogenous levels of nitric oxide under hypoxic conditions.

Hypoxia is a condition in which there is a decreased supply of oxygen to peripheral tissues. At least three classes of hypoxia exist and can be distinguished based on their root causes. Arterial hypoxia and anemic hypoxia are characterized by lower than normal oxygen tension (P_{O_2}) in arterial blood when the oxygen capacity and rate of blood flow are normal or even elevated. Arterial hypoxia results from exposure to pulmonary irritants that produce airway obstruction ranging from spasm or edema of the glottis to pulmonary edema (respiratory distress syndrome). Opioid narcotics and other drugs that depress respiration also produce arterial hypoxia. Anemic hypoxia results from decreased concentrations of functional hemoglobin, reduced numbers of red cells, or chemically induced alterations in hemoglobin. Stagnant (hypokinetic) hypoxia is characterized by a decreased rate of blood flow, as in heart failure and uncorrected vasodilation.

Pieper et al, *J. Pharmacol. Exp. Ther.*, 283(2):684-691 (1997), have shown that administering L-arginine *in vitro* can overcome a potential intracellular deficiency in nitric oxide production by diabetic endothelium. In the present invention, it was unexpectedly discovered and observed that administering N-hydroxy-L-arginine was more effective than L-arginine in the relaxation of aortic rings isolated from diabetic rats when compared to non-diabetic rats. The greater effectiveness of N-hydroxy-L-arginine to relax tissues isolated from diabetic species demonstrates that N-hydroxyguanidine compounds will be useful to treat clinical conditions associated with deficient nitric oxide pathways.

N-hydroxyguanidine compounds that are substrates for nitric oxide synthase have utility to treat essential hypertension, pulmonary hypertension,

pulmonary diseases (including respiratory distress syndrome, asthma, bronchitis/emphysema, and chronic obstructive pulmonary disease), circulatory hypoxia (including heart failure, strokes, and shock), specific organ hypoxia (in which decreased circulation to a specific organ resulting in localized circulatory hypoxia can be due to organic arterial obstruction or can result as a consequence of vasoconstriction, e.g., Reynauds Syndrome), localized hypoxia (which can result from venous obstruction and resultant congestions and reduced arterial blood inflow), edema (which increases the distance through which oxygen diffuses before it reaches the cells can also cause localized hypoxia), arterial diseases, central nervous system disorders, memory loss, and sexual dysfunctions (including hypoxic ischemia of the penis and female sexual dysfunctions). In addition, N-hydroxyguanidine compounds can be used to prevent and treat the same disorders that nitrovasodilators are now being clinically used to prevent and treat. Such nitrovasodilators include, but are not limited to, glyceryl trinitrate (also known as nitroglycerin) and erythrityl tetranitrate, and such disorders include, for example, cardiovascular disorders, such as myocardial ischemia, congestive heart failure, and angina pectoris.

As used herein, "sexual dysfunction" includes any sexual dysfunction in a patient. The patient can be male or female. Patient refers to animals, preferably mammals, more preferably humans. Sexual dysfunctions can include, for example, sexual desire disorders, sexual arousal disorders, orgasmic disorders and sexual pain disorders. Female sexual dysfunction refers to any female sexual dysfunction including, for example, sexual desire disorders, sexual arousal dysfunctions, orgasmic dysfunctions, sexual pain disorders, dyspareunia, and vaginismus. The female can be pre-menopausal or menopausal. Male sexual dysfunction refers to any male sexual dysfunction including, for example, male erectile dysfunction and impotence. In a preferred embodiment, "sexual dysfunctions" refer to sexual dysfunctions that are attributable to low oxygen conditions, including, but not limited to, sexual dysfunctions that are attributable to hypoxic ischemia, neuropathy, and arterial disease.

N-hydroxyguanidine compounds, including pharmaceutically acceptable salts thereof, can be administered with any pharmaceutically acceptable carrier.

The carrier should be pharmaceutically acceptable in the sense of being compatible with other ingredients of the formulation and not deleterious to the recipient thereof. Administration can be sublingual, oral, rectal, vaginal, intranasal, intraocular, topical, transdermal, parenteral, intraarterial, intravenous, buccal or by inhalation. The route of administration is at the discretion of the physician who takes into consideration the condition of the patient undergoing treatment. Formulations of N-hydroxyguanidine compounds can conveniently be presented in unit dosage forms and can be prepared by any of the methods known in the pharmaceutical arts.

10 N-hydroxyguanidine compounds that are substrates for nitric oxide synthase can be administered with other compounds, such as vasoactive agents. A vasoactive agent is any therapeutic agent that can relax vascular and non-vascular smooth muscle. Suitable vasoactive agents include, but are not limited to, long and short acting α -adrenergic blockers (such as, for example, phenoxybenzamine, dibenamine, doxazosin, terazosin, phentolamine, tolazoline, prazosin, trimazosin, yohimbine, moxislyte); calcium blockers (such as, for example, nifedipine, verapamil, diltiazem, gallopamil, niludipine, nimodipins, nicardipine); β -blockers (such as, for example, butixamine, dichloroisoproterenol, propanolol, alprenolol, bunolol, nadolol, oxprenolol, perbutolol, pinodolol, sotalol, timolol, metoprolol, atenolol, acebutolol, bevantolol, pafenolol, tolamodol); phosphodiesterase inhibitors (such as, for example, papaverine, zaprinast, sildenafil); adenosine, ergot alkaloids (such as, for example, ergotamine, ergotamine analogs, including, for example, acetergamine, brazergoline, bromerguride, cianergoline, delorgotril, disulergine, ergonovine maleate, ergotamine tartrate, etisulergine, lergotril, lysergide, mesulergine, metergoline, metergotamine, nicergoline, pergolide, propisergide, proterguride, terguride); vasoactive intestinal peptides (such as, for example, peptide histidine isoleucine, peptide histidine methionine, substance P, calcitonin gene-related peptide, neurokinin A, bradykinin, neurokinin B); dopamine agonists (such as, for example, apomorphine, bromocriptine, testosterone, cocaine, strychnine); opioid antagonists (such as, for example, naltrexone); prostaglandins (such as, for example, alprostadil, prostaglandin E₂, prostaglandin F₂, misoprostol, enprostil, arbaprostil,

unoprostone, trimoprostil, carboprost, limaprost, gemeprost, lantanoprost, ornoprostil, beraprost, sulprostone, rioprostil); endothelin antagonists (such as, for example, bosentan, sulfonamide endothelin antagonists, BQ-123, SQ 28608); potassium channel activators (such as, for example nicorandil, pinacidil, cromakalim) and mixtures thereof. Preferred are combinations of N-hydroxy-L-arginine with α -adrenergic antagonists, phosphodiesterase inhibitors, prostaglandins, dopamine agonists, potassium channel activators or endothelin antagonists.

When administered *in vivo*, the compounds and/or compositions of the invention can be administered in combination with pharmaceutically acceptable carriers and in dosages described herein. When the compositions of the present invention are administered as a mixture of at least one N-hydroxyguanidine compound and at least one vasoactive agent they can also be used in combination with one or more additional compounds which are known to be effective against the specific disease state targeted for treatment. N-hydroxyguanidine compounds can be administered simultaneously with, subsequently to, or prior to administration of the vasoactive agent(s) and/or other additional compound(s).

The compounds and/or compositions of the invention can be administered by any available and effective delivery systems including, but not limited to, orally, buccally, parenterally, by inhalation spray, by topical application, by injection into the corpus cavernosum tissue, by transurethral drug delivery, transdermally, rectally or vaginally in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles, as desired. Parenteral administration includes subcutaneous injections, intravenous, intramuscular, intrasternal injection, or infusion techniques. Transdermal drug administration, which is known to one skilled in the art, involves the delivery of pharmaceutical agents via percutaneous passage of the drug into the systemic circulation of the patient. Topical administration can also involve transdermal patches or iontophoresis devices. Other components can also be incorporated into the transdermal patches. For example, compositions and/or transdermal patches can be formulated with one or more preservatives or bacteriostatic agents including, for example, methyl hydroxybenzoate, propyl

hydroxybenzoate, chlorocresol, benzalkonium chloride, and the like.

5 Solid dosage forms for oral administration can include capsules, tablets, effervescent tablets, chewable tablets, pills, powders, granules and gels. In such solid dosage forms, the active compounds can be admixed with at least one inert
10 diluent such as sucrose, lactose or starch. Such dosage forms can also comprise, as in normal practice, additional substances other than inert diluents, e.g., lubricating agents, such as magnesium stearate. In the case of capsules, tablets, effervescent tablets, and pills, the dosage forms can also comprise buffering agents. Soft gelatin capsules can be prepared to contain a mixture of the active
15 compounds and/or compositions of the invention and vegetable oil. Hard gelatin capsules can contain granules of the active compounds and/or compositions in combination with a solid, pulverulent carrier such as lactose, saccharose, sorbitol, mannitol, potato starch, corn starch, amylopectin, cellulose derivatives of gelatin. Tablets and pills can be prepared with enteric coatings.

20 Liquid dosage forms for oral administration can include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions can also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

25 Dosage forms for topical administration of the compounds and/or compositions of the invention can include creams, sprays, lotions, gels, ointments, coatings for condoms, and the like. Administration of the cream or gel can be accompanied by use of an applicator or by transurethral drug delivery using a syringe with or without a needle or penile or vaginal insert or device, and is
30 within the skill of the art. Typically a lubricant and/or a local anesthetic for desensitization can also be included in the formulation or provided for use as needed. Lubricants include, for example, K-Y jelly (available from Johnson & Johnson) or a lidocaine jelly, such as Xylocaine 2% jelly (available from Astra Pharmaceutical Products). Local anesthetics include, for example, novocaine, procaine, tetracaine, benzocaine and the like.

The compounds and/or compositions of the invention will typically be administered in a pharmaceutical composition containing one or more selected

carriers or excipients. Suitable carriers include, for example, water, silicone, waxes, petroleum jelly, polyethylene glycol, propylene glycol, liposomes, sugars, and the like. The compositions can also include one or more permeation enhancers including, for example, dimethylsulfoxide (DMSO), dimethyl formamide (DMF), N,N-dimethyl-acetamide (DMA), decylmethylsulfoxide (C10MSO), polyethylene glycol monolaurate (PEGML), glycerol monolaurate, lecithin, 1-substituted azacycloheptan-2-ones, particularly 1-N-dodecylcyclazacycloheptan-2-ones (available under the trademark Azone from Nelson Research & Development Co., Irvine, CA), alcohols and the like.

Suppositories for rectal or vaginal administration of the compounds and/or compositions of the invention can be prepared by mixing the drug with a suitable nonirritating excipient such as cocoa butter and polyethylene glycols which are solid at room temperature but liquid at rectal or vaginal temperature, such that they will melt in the rectum or vagina and release the drug.

Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions can be formulated according to the known art using suitable dispersing agents, wetting agents and/or suspending agents. The sterile injectable preparation can also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Acceptable vehicles and solvents that can be used include, for example, water, Ringer's solution, and isotonic sodium chloride solution. Sterile fixed oils are also conventionally used as a solvent or suspending medium.

The compounds and/or compositions of the invention can be formulated in neutral or acid salt forms. Such pharmaceutically acceptable salts include, for example, those formed with free amino groups such as those derived from hydrochloric, hydrobromic, hydroiodide, phosphoric, sulfuric, acetic, trifluoroacetic, citric, benzoic, fumaric, glutamic, lactic, malic, maleic, succinic, tartaric, p-toluenesulfonic, methanesulfonic acids, gluconic acid, glycolic acid and the like, and those formed with free carboxyl groups, such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, and the like. Preferred pharmaceutically acceptable salts of N-hydroxy-L-arginine are hydrochloride,

glutamate, butyrate, glycolate, trifluoroacetate and acetate, and the most preferred salts are the acetate or trifluoroacetate salts.

"Therapeutically effective amount" refers to the amount of the N-hydroxyguanidine compounds or vasoactive agents that is effective to achieve its intended purpose. While individual patient needs may vary, determination of optimal ranges for effective amounts of N-hydroxyguanidine compounds and vasoactive agents is within the skill of the art. Generally, the dosage required to provide an effective amount of the compound and/or composition, and which can be adjusted by one of ordinary skill in the art will vary, depending on the age, health, physical condition, sex, weight, extent of the dysfunction of the recipient, frequency of treatment and the nature and scope of the dysfunction.

The amount of a given vasoactive agent which will be effective in the treatment of a particular dysfunction or condition will depend on the nature of the dysfunction or condition, and can be determined by standard clinical techniques, including reference to Goodman and Gilman, *The Pharmacological Basis of Therapeutics* (9th Edition, 1995); *The Physician's Desk Reference* (49th Ed.); Medical Economics (1995); *Drug Facts and Comparisons* (1993); and *The Merck Index* (12th Ed.), Merck & Co., Inc. (1996), the disclosures of each of which are incorporated herein by reference in their entirety. The precise dose to be used in the formulation will also depend on the route of administration, and the seriousness of the dysfunction or disorder, and should be decided by the physician and the patient's circumstances.

The usual dose of N-hydroxy-L-arginine administered to a patient is about 0.25 g/day to about 10 g/day, preferably about 2 g/day to about 4 g/day, more preferably about 3 g/day. The dose can optionally be administered orally at least one hour prior to sexual activity or sexual intercourse. Effective doses can be extrapolated from dose-response curves derived from *in vitro* or animal model test systems and are in the same ranges or less than those described for other commercially available compounds in, for example, the *Physician's Desk Reference* (49th Ed.).

The dosage regimen for treating a condition with the compounds and/or compositions of the invention is selected in accordance with a variety of factors,

including the type, age, weight, sex, diet and medical condition of the patient, the severity of the dysfunction, the route of administration, pharmacological considerations such as the activity, efficacy, pharmacokinetic and toxicology profiles of the particular compound used, whether a drug delivery system is used, and whether the compound is administered as part of a drug combination. Thus, the dosage regimen actually used can vary widely and therefore may deviate from the preferred dosage regimen set forth above.

Particularly preferred methods of administering the N-hydroxyguanidine compounds and/or compositions for the treatment of male sexual dysfunctions are oral, buccal, transdermal application, by injection into the corpus cavernosum, by transurethral administration, by inhalation or by the use of suppositories. The preferred methods of administration for female sexual dysfunctions are oral, buccal, topical application, transdermal application, by inhalation or by the use of suppositories.

The present invention also provides pharmaceutical kits comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compounds and/or compositions of the present invention, including, one or more N-hydroxyguanidine compounds and, optionally, one or more of the vasoactive agents described herein. Such kits can also include, for example, other compounds and/or compositions (e.g., permeation enhancers, lubricants), a device(s) for administering the compounds and/or compositions, and written instructions in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which instructions can also reflect approval by the agency of manufacture, use or sale for human administration.

EXAMPLES

The following examples are for purposes of illustration, and are not intended to limit the scope of the specification or claims.

The utility of N-hydroxy-L-arginine, a N-hydroxyguanidine compound, to promote the synthesis of NO was demonstrated in rabbit corpus cavernosum tissue (the erectile tissue of the penis) and rat aortic rings. Normal arterial oxygen tension measures as approximately 75-100 mm Hg, and normal venous

oxygen tension measures as approximately 30-40 mm Hg. Experimentally equivalent oxygen concentrations for tissue culture are 20% oxygen (referred to herein as "normoxic"), which corresponds to approximately 140 mm Hg, to simulate arterial oxygen tension, and 5% oxygen (referred to herein as "hypoxic"), which corresponds to approximately 35 mm Hg, to simulate venous oxygen tension. Lower oxygen concentrations were also used as indicated. The preparation of the tissues for study was carried out as described herein.

Example 1: Preparation of corpus cavernosum tissue

Rabbits were euthanized with an overdose of intravenous pentobarbital (60 mg/kg) and immediately exsanguinated. The entire penis was then removed and the corpus cavernosum tissue dissected free from the surrounding tunica albuginea and cut into strips (3x3x7mm). Corpus cavernosum tissues were maintained at 4-6°C in M-400 solution (composition per 100 ml: mannitol, 4.19 g; KH_2PO_4 , 0.205 g; $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, 0.97 g; KCl, 0.112 g; NaHCO_3 , 0.084 g) until used. Corpus cavernosum tissues were typically used between 2 and 16 hours from extraction.

Corpus cavernosum tissues, prepared as described above, were subjected to the following tests to demonstrate that N-hydroxy-L-arginine is useful to promote the synthesis of nitric oxide even under conditions of low oxygen tension.

Example 2: Basal and acetylcholine stimulated production of cGMP by corpus cavernosum tissues

Measurement of cyclic GMP in corpus cavernosal tissues was carried out as follows. Corpus cavernosal tissue strips were immersed in a 10 ml organ chamber containing a physiological salt solution, maintained at 37°C and aerated with 5% CO_2 /95% air, pH 7.4. Each strip was incrementally stretched to optimal isometric tension, as determined by maximal contractile responses to 1 μM phenylephrine ((R)-3-hydroxy- α -[(methylamino)methyl]-benzenemethanol hydrochloride). The physiological salt solution was then replaced with one bubbled with either 20% oxygen or 0% oxygen and the tissues were then given 0.5 μM phenylephrine, 30 μM Zaprinast and 100 μM IBMX (3-isobutyl-1-methylxanthine, cAMP specific phosphodiesterase) and incubated for 15 minutes; after which time each tissue was incubated with the test drug (or control drug) at various concentrations or

with vehicle (the buffer in which the drugs are delivered). Tissues were incubated for another 5 minutes then immediately frozen in liquid nitrogen and stored at -80°C until extraction for cyclic nucleotide assay. Tissues were extracted by homogenization in 6% trichloroacetic acid followed by ether (H₂O-saturated) extraction and lyophilization. cGMP levels were determined by ELISA using a kit from Cayman Chemical Co. (Ann Arbor, MI).

Protein concentration in the corpus cavernosum tissue was determined using the Bio-Rad Protein Assay Kit microtiter plate assay procedure (Bio-Rad, Hercules, CA) with bovine serum albumin as the standard.

The basal level cGMP production by corpus cavernosum tissues under normoxic conditions was measured in the presence of vehicle, L-arginine and N-hydroxy-L-arginine. The measured concentrations of cGMP in picomoles were normalized based on milligrams of protein to provide a valid comparison between each tissue sample. As depicted in Fig. 1, under normoxic conditions the corpus cavernosum tissues are capable of producing cGMP without the addition of a drug or other stimulating agent (Control). Addition of 300 μ M L-arginine or 30 μ M N-hydroxy-L-arginine stimulated cGMP production approximately equally. Addition of 300 μ M N-hydroxy-L-arginine, however, was effective to stimulate a higher concentration of cGMP production, and the increased amount of cGMP production was demonstrated to be statistically significant.

The level of cGMP production by corpus cavernosum tissues stimulated with acetylcholine was examined under conditions of normoxia and hypoxia (in this experiment, 0% oxygen), in the presence of L-arginine and N-hydroxy-L-arginine. The measured concentrations of cGMP in picomoles were normalized based on milligrams of protein to provide a valid comparison between each tissue sample. As depicted in Figs. 2A and 2B, cGMP production was greatly reduced under conditions of low oxygen tension, in this case severe hypoxia of 0% oxygen. Comparable to the results depicted in Fig. 1, the results presented in Figs. 2A and 2B show that under normoxia conditions (20% oxygen) and hypoxia, the addition of N-hydroxy-L-arginine results in a statistically significant increase in the concentration of cGMP compared to the basal values. Under the same conditions of normoxia and hypoxia, the administration of L-arginine was not effective to

statistically increase the concentration of cGMP compared to the basal values. Thus, the administration of N-hydroxy-L-arginine has been demonstrated to be useful to promote the synthesis of nitric oxide even under conditions of low oxygen tension, in this case severe hypoxia of 0% oxygen.

5 **Example 3: Preparation of corpus cavernosum tissues for relaxation studies**

Corpus cavernosum tissue was prepared as described in Example 1. For experimentation to measure relaxation of the tissue under various conditions, the corpus cavernosum tissues were then suspended in 10 ml organ chambers and
10 bathed in a physiological salt solution, maintained at 37°C and aerated with 5% CO₂/95% air, pH 7.4. Each corpus cavernosum tissue strip was incrementally stretched to optimal isometric tension as determined by the maximal contractile response to 1 μ M phenylephrine. After several exchanges of fresh physiological solution, the media was bubbled with gas mixtures containing 5% CO₂, the
15 indicated amount of oxygen (between 0% and 20%) and the remaining percentage was N₂, for thirty minutes. Corpus cavernosum tissues were then contracted with a submaximal concentration of phenylephrine, and the contraction was allowed to reach a steady state. Once a steady state of muscle tension was reached, the tissue was exposed to various concentrations of test compounds, and the relaxation
20 responses were recorded. Relaxation responses were expressed as a percentage of total relaxation induced by a supramaximal addition of papaverine (total loss of tone) at the end of the experiment. The data in Figs. 3-6 are expressed as mean \pm standard mean error.

25 **Example 4: Inhibition of P450 does not inhibit N-hydroxy-L-arginine induced relaxation of corpus cavernosum tissue**

The tissues were prepared according to Example 3 under normoxic conditions (20% oxygen). The percent relaxation of corpus cavernosum tissue induced by N-hydroxy-L-arginine in the presence of miconazole (an inhibitor of P450) was measured to determine if nitric oxide relaxation occurs via the P450
30 pathway. As can be seen from Fig. 3, the addition of miconazole to corpus cavernosum tissues in the presence of increasing concentrations of N-hydroxy-L-arginine does not significantly effect the relaxation of the tissues. The addition of L-NNA (N^G-nitro-L-arginine, an inhibitor of nitric oxide synthase), however, does

decrease the effectiveness of N-hydroxy-L-arginine to relax the tissue. Thus, nitric oxide relaxation of corpus cavernosum tissue does not proceed via the P450 pathway.

5 **Example 5: Inhibition of nitric oxide synthase inhibits N-hydroxy-L-arginine induced relaxation of corpus cavernosum tissue**

10 The tissues were prepared according to Example 3 under normoxic conditions (20% oxygen). The percent relaxation of corpus cavernosum tissue induced by N-hydroxy-L-arginine in the presence of two different inhibitors of corporal tissue relaxation, L-NNA (N^G-nitro-L-arginine, a nitric oxide synthase inhibitor) and ODQ (1H-[1,2,4]oxadiazolo[4,3,a]quinoxalin-1-one, an inhibitor of soluble guanylate cyclase), was measured in order to demonstrate that N-hydroxy-L-arginine is converted to nitric oxide and citrulline by the action of nitric oxide synthase. As can be seen from Fig. 4A, the addition of either L-NNA or ODQ significantly decreased the measured relaxation of the corpus cavernosum tissue even in the presence of increasing concentrations of N-hydroxy-L-arginine. Thus, the synthesis of nitric oxide from N-hydroxy-L-arginine is catalyzed by nitric oxide synthase.

15 In order to determine the specificity of the induced relaxation, the same experiment was repeated using varying concentrations of hydroxylamine instead of N-hydroxy-L-arginine. As seen in Fig. 4B, the addition of ODQ significantly decreased the relaxation of the corpus cavernosum tissue in the presence of increasing concentrations of hydroxylamine, while L-NNA had no effect. Thus, hydroxylamine induces the relaxation of the tissue by a guanylate cyclase mediated pathway and nitric oxide synthase is not involved in the process.

25 **Example 6: Relaxation of corpus cavernosum tissue under normoxic and hypoxic conditions**

30 The tissues were prepared according to Example 3 under normoxic conditions (20% oxygen) or hypoxic conditions (5% oxygen) as indicated herein. Corpus cavernosum tissue was stimulated to relax either under normoxic or hypoxic conditions by the administration of acetylcholine. After the tissue was allowed to partially recover, it was again stimulated to relax by administering various concentrations of either L-arginine or N-hydroxy-L-arginine (arrow marked "drugs" in Figs. 5A and 5B). Acetylcholine is a preganglionic

neurotransmitter which stimulates relaxation of vascular tissues by stimulating endogenous nitric oxide production through the nitric oxide synthase pathway. Thus, intracellular stores of L-arginine and N-hydroxy-L-arginine are depleted following stimulation of the tissues with acetylcholine. As shown in Figs. 5A and 5B, concentrations of N-hydroxy-L-arginine of 50 μ M or greater was sufficient to induce relaxation of corpus cavernosum tissues under both normoxic and hypoxic conditions, and increasing concentrations N-hydroxy-L-arginine were increasingly effective. In contrast, concentrations of L-arginine as high as 1 mM were not effective to induce relaxation of corpus cavernosum tissues. These results demonstrate that N-hydroxy-L-arginine, but not L-arginine, can be taken into a cell and can then be converted to nitric oxide to induce the relaxation of the tissue even under hypoxic conditions.

Example 7: Relaxation of corpus cavernosum tissues under various oxygen concentrations by N-hydroxy-L-arginine

Corpus cavernosum tissue samples were prepared as described in Example 3 under the following oxygen concentrations: 0%, 1%, 2%, 5% and 20%. At oxygen concentrations of 0%, 1%, 2% and 5%, the administration of 1 mM L-arginine to the tissue had no measurable effect on tissue relaxation (data not shown). Administration of 1 mM N-hydroxy-L-arginine to the tissue at all oxygen concentrations resulted in significant relaxation of the tissue (Fig. 6). Thus, N-hydroxy-L-arginine has been shown to be effective to induce the synthesis of cGMP under hypoxic conditions (Example 2), and to be effective to induce measurable relaxation of corpus cavernosum tissue samples.

Example 8: Preparation of rat aortic segments

Diabetes was induced in rats by administering a single injection of streptozotocin (60 mg/kg; i.p.). Diabetic rats remained untreated for a total of 8 weeks. Non-diabetic rats were those that did not receive the streptozotocin treatment.

Rats were anesthetized with diethyl ether and exsanguinated. The thoracic aortae was then removed and the tissue dissected free from the surrounding periadventitial fat and connective tissue, taking precautions to avoid touching the luminal surface. The tissue was cut into 8 segments, each approximately 3-4 mm

in length. Rat aortic segments were maintained at 4-6 °C in a Krebs-bicarbonate buffer (composition per millimolar: NaCl, 120; KCl, 5.6; MgCl₂, 1.2; NaH₂PO₄, 1.2; dextrose, 10; NaHCO₃, 25; CaCl₂, 2.5; pH 7.4) until used. Aortic segments were typically used between 2 and 16 hours from extraction.

5 For experimentation to measure relaxation of the tissue under various conditions, the rat aortic segments were suspended in 10 ml organ chambers and bathed in a physiological salt solution, maintained at 37°C and aerated with 5% CO₂/95% air, pH 7.4. Each aortic tissue strip was submitted to 1.5 g of resting tension. After several exchanges of fresh physiological solution, the media was
10 bubbled with gas mixtures containing 5% CO₂, the indicated amount of oxygen (between 0% and 20%), and the remaining percentage was N₂, for thirty minutes. The aortic segments were then contracted with submaximal concentration of norepinephrine and the contraction was allowed to reach a steady state. Once a steady state of muscle tension was reached, the tissue was exposed to various
15 concentrations of test compounds and the relaxation responses were recorded. Relaxation responses were expressed as a percentage of total relaxation induced by a supramaximal addition of papaverine (total loss of tone) at the end of the experiment. In Figs. 7-12, the data are expressed as mean ± standard mean error.

Aortic segments prepared as described herein, were subjected to the
20 following tests to demonstrate that N-hydroxy-L-arginine is useful to promote the synthesis of nitric oxide even under conditions of low oxygen tension.

Example 9: L-arginine and N-hydroxy-L-arginine induced relaxation of non-diabetic and diabetic aorta segments

The tissues were prepared according to Example 8 under normoxic
25 conditions (95% oxygen). The percent contraction of aortic segments from non-diabetic and diabetic rats induced by L-arginine and N-hydroxy-L-arginine was measured. Figs. 7A and 7B show that N-hydroxy-L-arginine was more effective in inducing relaxation in both non-diabetic and diabetic rats. Fig. 8 is a direct comparison of the N-hydroxy-L-arginine induced relaxation response of aortic
30 segments obtained from non-diabetic and diabetic rats. At 1 μM N-hydroxy-L-arginine, the observed effect was significantly different and shows that N-hydroxy-L-arginine was more effective in inducing the relaxation of aortic

segments obtained from diabetic rats.

Example 10: Inhibition of nitric oxide synthase inhibits N-hydroxy-L-arginine induced relaxation of aorta segments isolated from non-diabetic and diabetic rats

5 The tissues were prepared according to Example 8 under normoxic conditions (20% oxygen). The percent contraction of rat aortic segments induced by N-hydroxy-L-arginine in the presence of a nitric oxide synthase inhibitor, L-NAME (N^G-nitro-L-arginine, 10 μ M), was measured in order to demonstrate that N-hydroxy-L-arginine is converted to nitric oxide and citrulline by the action of nitric
10 oxide synthase. As can be seen from Figs. 9A and 9B, the addition of L-NAME significantly decreased the measured relaxation of the aortic segments obtained from non-diabetic and diabetic rats, respectively, even in the presence of increasing concentrations of N-hydroxy-L-arginine. Thus, the synthesis of nitric oxide from N-hydroxy-L-arginine is catalyzed by nitric oxide synthase.

15 **Example 11: Acetylcholine induced relaxation of aortic segments isolated from non-diabetic and diabetic rats**

The tissues were prepared according to Example 8 under normoxic conditions (95% oxygen). The percent contraction of rat aortic segments induced by increasing concentrations of ACh in the presence of either 10 μ M L-arginine or
20 10 μ M N-hydroxy-L-arginine was measured to demonstrate that N-hydroxy-L-arginine is more effective in inducing the relaxation of rings isolated from diabetic rats. As can be seen from Fig. 10A, neither L-arginine nor N-hydroxy-L-arginine in the presence of 10 nM to 10 μ M ACh resulted in any change in the relaxation of the aortic segments isolated from non-diabetic rats. In contrast, N-hydroxy-L-
25 arginine, in the presence of 10 nM to 10 μ M ACh, increased the relaxation of rat aortic segments isolated from diabetic rats (Fig. 10B). Under the same conditions, L-arginine resulted in a decreased relaxation. These results demonstrate that N-hydroxy-L-arginine can be taken up into a cell more efficiently, and can then be converted to nitric oxide to induce the relaxation of the tissue isolated from
30 diabetic rats.

Example 12: Relaxation of aortic segments under normoxic and hypoxic conditions

The tissues were prepared according to Example 8 under two normoxic

conditions (95% and 20% oxygen) or hypoxic conditions (5% oxygen) as indicated herein. Aortic segments from non-diabetic rats were stimulated to relax under either normoxic or hypoxic conditions by administering acetylcholine. After the tissue was allowed to partially recover, it was again stimulated to relax by
5 administering various concentrations of either L-arginine or N-hydroxy-L-arginine (arrow marked "drugs" in Figs. 11A, 11B and 11C). Acetylcholine is a preganglionic neurotransmitter which stimulates relaxation of vascular tissues by stimulating endogenous nitric oxide production through the nitric oxide synthase pathway. Thus intracellular stores of L-arginine and N-hydroxy-L-arginine are
10 depleted following stimulation of the tissues with acetylcholine. As shown in Figs. 11A, 11B and 11C, concentrations of N-hydroxy-L-arginine of 10 μ M or greater were sufficient to induce relaxation of aortic segments under both normoxic and hypoxic conditions, and increasing concentrations of N-hydroxy-L-arginine were increasingly effective. In contrast, concentrations of L-arginine as
15 high as 1 mM were not effective to induce the relaxation of aortic segments. These results demonstrate that N-hydroxy-L-arginine, and not L-arginine, can be taken up into a cell and can then be converted to nitric oxide to induce the relaxation of the tissue even under hypoxic conditions.

Example 13: Vasoactive response of anesthetized autoperfused rats

20 The left hindlimb of the anesthetized rats were perfused through the femoral artery at a constant rate of 3.3 ml/min/kg with blood from the carotid artery using a peristaltic pump. The vasoactive response induced by the bolus infusion of increasing concentrations of L-arginine or N-hydroxy-L-arginine into the left hindlimb of autoperfused non-diabetic or diabetic rats was determined by
25 measuring the change in the perfusion pressure, which was previously elevated by a norepinephrine constant infusion (2 μ g/min/kg). Fig. 12A shows the perfusion pressures resulting from the administration of 0.1 mg/kg to 10 mg/kg L-arginine to either non-diabetic or diabetic rats. At all concentrations of L-arginine, the pressure change was greater for the non-diabetic rats when compared to the
30 diabetic rats. Fig 12B shows that there was no difference in the perfusion pressure when increasing concentrations of N-hydroxy-L-arginine were administered to non-diabetic and diabetic rats.

The disclosure of each patent, patent application and publication cited or described in the specification is incorporated by reference herein in its entirety.

Although the invention has been set forth in detail, one skilled in the art will appreciate that numerous changes and modifications can be made to the
5 invention without departing from the spirit and scope thereof.

CLAIMS

What is claimed is:

1. A method of promoting synthesis of nitric oxide or endothelium-derived relaxing factor in hypoxic mammalian tissue comprising administering to a patient in need thereof a therapeutically effective amount of at least one N-hydroxyguanidine compound.
2. The method of claim 1, wherein the N-hydroxyguanidine compound is N-aryl-N'-hydroxyguanidine, a nitrosated N-aryl-N'-hydroxyguanidine, a nitrosylated N-aryl-N'-hydroxyguanidine, N-hydroxy-L-arginine, or an analog of N-hydroxy-L-arginine.
3. The method of claim 2, wherein the N-hydroxyguanidine compound is N-hydroxy-L-arginine.
4. The method of claim 2, wherein the analog of N-hydroxy-L-arginine is N^ω-hydroxy-homo-L-arginine, a carboxylic ester of N-hydroxy-L-arginine, a N- α derivative of N-hydroxy-L-arginine, N^G-hydroxy-agmatine, N^G-hydroxy-L-argininic acid, a nitrosated N-hydroxy-L-arginine, a nitrosylated N-hydroxy-L-arginine, a nitrosated N^ω-hydroxy-homo-L-arginine, a nitrosylated N^ω-hydroxy-homo-L-arginine, a nitrosated carboxylic ester of N-hydroxy-L-arginine, a nitrosylated carboxylic ester of N-hydroxy-L-arginine, a nitrosated N- α derivative of N-hydroxy-L-arginine, a nitrosylated N- α derivative of N-hydroxy-L-arginine, a nitrosated N^G-hydroxy-agmatine, a nitrosylated N^G-hydroxy-agmatine, a nitrosated N^G-hydroxy-L-argininic acid, or a nitrosylated N^G-hydroxy-L-argininic acid.
5. The method of claim 4, wherein the analog of N-hydroxy-L-arginine is the nitrosated N-hydroxy-L-arginine or the nitrosylated N-hydroxy-L-arginine.
6. The method of claim 5, wherein the nitrosated N-hydroxy-L-arginine or the nitrosylated N-hydroxy-L-arginine is an adduct of N-hydroxy-L-arginine with nitric oxide.
7. The method of claim 1, wherein the hypoxic mammalian tissue is attributable to a pulmonary disease, a cardiovascular disorder, circulatory hypoxia, specific organ hypoxia, localized hypoxia, edema, a central nervous system disorder, memory loss, or arterial disease.
8. The method of claim 1, wherein the N-hydroxyguanidine compound

is administered orally, parenterally, topically, vaginally, by inhalation, or by transurethral application.

9. The method of claim 1, wherein the N-hydroxyguanidine compound is a substrate for nitric oxide synthase.

10. The method of claim 1, further comprising administering to the patient a therapeutically effective amount of at least one vasoactive agent.

11. The method of claim 10, wherein the vasoactive agent is an α -blocker, a calcium blocker, a β -blocker, a phosphodiesterase inhibitor, adenosine, an ergot alkaloid, a vasoactive intestinal peptide, a dopamine agonist, an opioid antagonist, a prostaglandin, an endothelin antagonist, a potassium channel activator, or a mixture thereof.

12. A method of promoting relaxation of vascular and non-vascular smooth muscle in mammalian tissue under low oxygen conditions comprising administering to a patient in need thereof a therapeutically effective amount of at least one N-hydroxyguanidine compound.

13. The method of claim 12, wherein the N-hydroxyguanidine compound is N-aryl-N'-hydroxyguanidine, a nitrosated N-aryl-N'-hydroxyguanidine, a nitrosylated N-aryl-N'-hydroxyguanidine, N-hydroxy-L-arginine, or an analog of N-hydroxy-L-arginine.

14. The method of claim 13, wherein the N-hydroxyguanidine compound is N-hydroxy-L-arginine.

15. The method of claim 13, wherein the analog of N-hydroxy-L-arginine is N^ω-hydroxy-homo-L-arginine, a carboxylic ester of N-hydroxy-L-arginine, a N- α derivative of N-hydroxy-L-arginine, N^G-hydroxy-agmatine, N^G-hydroxy-L-argininic acid, a nitrosated N-hydroxy-L-arginine, a nitrosylated N-hydroxy-L-arginine, a nitrosated N^ω-hydroxy-homo-L-arginine, a nitrosylated N^ω-hydroxy-homo-L-arginine, a nitrosated carboxylic ester of N-hydroxy-L-arginine, a nitrosylated carboxylic ester of N-hydroxy-L-arginine, a nitrosated N- α derivative of N-hydroxy-L-arginine, a nitrosylated N- α derivative of N-hydroxy-L-arginine, a nitrosated N^G-hydroxy-agmatine, a nitrosylated N^G-hydroxy-agmatine, a nitrosated N^G-hydroxy-L-argininic acid, or a nitrosylated N^G-hydroxy-L-argininic acid.

16. The method of claim 15, wherein the analog of N-hydroxy-L-arginine

is the nitrosated N-hydroxy-L-arginine or the nitrosylated N-hydroxy-L-arginine.

17. The method of claim 16, wherein the nitrosated N-hydroxy-L-arginine or the nitrosylated N-hydroxy-L-arginine is an adduct of N-hydroxy-L-arginine with nitric oxide.

18. The method of claim 12, wherein the N-hydroxyguanidine compound is administered orally, parenterally, topically, vaginally, by inhalation, or by transurethral application.

19. The method of claim 12, wherein the N-hydroxyguanidine compound is a substrate for nitric oxide synthase.

20. The method of claim 12, further comprising administering to the patient at least one vasoactive agent.

21. The method of claim 20, wherein the vasoactive agent is an α -blocker, a calcium blocker, a β -blocker, a phosphodiesterase inhibitor, adenosine, an ergot alkaloid, a vasoactive intestinal peptide, a dopamine agonist, an opioid antagonist, a prostaglandin, an endothelin antagonist, a potassium channel activator or a mixture thereof.

22. A method of treating a sexual dysfunction in a patient in need thereof comprising administering to the patient a therapeutically effective amount of at least one N-hydroxyguanidine compound.

23. The method of claim 22, wherein the N-hydroxyguanidine compound is N-aryl-N'-hydroxyguanidine, a nitrosated N-aryl-N'-hydroxyguanidine, a nitrosylated N-aryl-N'-hydroxyguanidine, N-hydroxy-L-arginine, or an analog of N-hydroxy-L-arginine.

24. The method of claim 23, wherein the N-hydroxyguanidine compound is N-hydroxy-L-arginine.

25. The method of claim 23, wherein the analog of N-hydroxy-L-arginine is N^ω-hydroxy-homo-L-arginine, a carboxylic ester of N-hydroxy-L-arginine, a N- α derivative of N-hydroxy-L-arginine, N^G-hydroxy-argmatine, N^G-hydroxy-L-argininic acid, a nitrosated N-hydroxy-L-arginine, a nitrosylated N-hydroxy-L-arginine, a nitrosated N^ω-hydroxy-homo-L-arginine, a nitrosylated N^ω-hydroxy-homo-L-arginine, a nitrosated carboxylic ester of N-hydroxy-L-arginine, a nitrosylated carboxylic ester of N-hydroxy-L-arginine, a nitrosated N- α derivative

of N-hydroxy-L-arginine, a nitrosylated N- α derivative of N-hydroxy-L-arginine, a nitrosated N^G-hydroxy-arginine, a nitrosylated N^G-hydroxy-arginine, a nitrosated N^G-hydroxy-L-argininic acid, or a nitrosylated N^G-hydroxy-L-argininic acid.

26. The method of claim 25, wherein the analog of N-hydroxy-L-arginine is the nitrosated N-hydroxy-L-arginine or the nitrosylated N-hydroxy-L-arginine.

27. The method of claim 26, wherein the nitrosated N-hydroxy-L-arginine or the nitrosylated N-hydroxy-L-arginine is an adduct of N-hydroxy-L-arginine with nitric oxide.

28. The method of claim 22, wherein the N-hydroxyguanidine compound is a substrate for nitric oxide synthase.

29. The method of claim 22, wherein the sexual dysfunction is attributable to low oxygen conditions.

30. The method of claim 22, wherein the sexual dysfunction is attributable to hypoxic ischemia.

31. The method of claim 22, wherein the sexual dysfunction is attributable to neuropathy.

32. The method of claim 22, wherein the sexual dysfunction is attributable to arterial disease.

33. The method of claim 22, wherein the patient is male.

34. The method of claim 22, wherein the patient is female.

35. The method of claim 22, wherein the N-hydroxyguanidine compound is administered orally, parenterally, topically, vaginally, by inhalation, or by transurethral application.

36. The method of claim 22, further comprising administering to the patient at least one vasoactive agent.

37. The method of claim 36, wherein the vasoactive agent is an α -blocker, a calcium blocker, a β -blocker, a phosphodiesterase inhibitor, adenosine, an ergot alkaloid, a vasoactive intestinal peptide, a dopamine agonist, an opioid antagonist, a prostaglandin, an endothelin antagonist, a potassium channel activator or a mixture thereof.

38. A method of promoting synthesis of nitric oxide or endothelium-derived relaxing factor in mammalian tissue, wherein the mammalian tissue has a

deficient nitric oxide pathway, comprising administering to a patient in need thereof a therapeutically effective amount of at least one N-hydroxyguanidine compound.

39. The method of claim 38, wherein the deficient nitric oxide pathway is attributable to diabetes.

40. The method of claim 38, wherein the N-hydroxyguanidine compound is N-aryl-N'-hydroxyguanidine, a nitrosated N-aryl-N'-hydroxyguanidine, a nitrosylated N-aryl-N'-hydroxyguanidine, N-hydroxy-L-arginine, or an analog of N-hydroxy-L-arginine.

41. The method of claim 40, wherein the N-hydroxyguanidine compound is N-hydroxy-L-arginine.

42. The method of claim 40, wherein the analog of N-hydroxy-L-arginine is N^ω-hydroxy-homo-L-arginine, a carboxylic ester of N-hydroxy-L-arginine, a N-α derivative of N-hydroxy-L-arginine, N^G-hydroxy-agmatine, N^G-hydroxy-L-argininic acid, a nitrosated N-hydroxy-L-arginine, a nitrosylated N-hydroxy-L-arginine, a nitrosated N^ω-hydroxy-homo-L-arginine, a nitrosylated N^ω-hydroxy-homo-L-arginine, a nitrosated carboxylic ester of N-hydroxy-L-arginine, a nitrosylated carboxylic ester of N-hydroxy-L-arginine, a nitrosated N-α derivative of N-hydroxy-L-arginine, a nitrosylated N-α derivative of N-hydroxy-L-arginine, a nitrosated N^G-hydroxy-agmatine, a nitrosylated N^G-hydroxy-agmatine, a nitrosated N^G-hydroxy-L-argininic acid, or a nitrosylated N^G-hydroxy-L-argininic acid.

43. The method of claim 42, wherein the analog of N-hydroxy-L-arginine is the nitrosated N-hydroxy-L-arginine or the nitrosylated N-hydroxy-L-arginine.

44. The method of claim 43, wherein the nitrosated N-hydroxy-L-arginine or the nitrosylated N-hydroxy-L-arginine is an adduct of N-hydroxy-L-arginine with nitric oxide.

45. The method of claim 38, wherein the N-hydroxyguanidine compound is a substrate for nitric oxide synthase.

46. The method of claim 38, wherein the N-hydroxyguanidine compound is administered orally, parenterally, topically, vaginally, by inhalation, or by transurethral application.

47. The method of claim 38, further comprising administering to the

patient at least one vasoactive agent.

48. The method of claim 47, wherein the vasoactive agent is an α -blocker, a calcium blocker, a β -blocker, a phosphodiesterase inhibitor, adenosine, an ergot alkaloid, a vasoactive intestinal peptide, a dopamine agonist, an opioid antagonist, a prostaglandin, an endothelin antagonist, a potassium channel activator or a mixture thereof.

49. A pharmaceutical composition comprising at least one N-hydroxyguanidine compound and a pharmaceutically acceptable carrier.

50. The pharmaceutical composition of claim 49, wherein the N-hydroxyguanidine compound is N-aryl-N'-hydroxyguanidine, a nitrosated N-aryl-N'-hydroxyguanidine, a nitrosylated N-aryl-N'-hydroxyguanidine, N-hydroxy-L-arginine, or an analog of N-hydroxy-L-arginine.

51. The pharmaceutical composition of claim 50, wherein the N-hydroxyguanidine compound is N-hydroxy-L-arginine.

52. The pharmaceutical composition of claim 50, wherein the analog of N-hydroxy-L-arginine is a N^ω-hydroxy-homo-L-arginine, a carboxylic ester of N-hydroxy-L-arginine, a N- α derivative of N-hydroxy-L-arginine, N^G-hydroxy-agmatine, N^G-hydroxy-L-argininic acid, a nitrosated N-hydroxy-L-arginine, a nitrosylated N-hydroxy-L-arginine, a nitrosated N^ω-hydroxy-homo-L-arginine, a nitrosylated N^ω-hydroxy-homo-L-arginine, a nitrosated carboxylic ester of N-hydroxy-L-arginine, a nitrosylated carboxylic ester of N-hydroxy-L-arginine, a nitrosated N- α derivative of N-hydroxy-L-arginine, a nitrosylated N- α derivative of N-hydroxy-L-arginine, a nitrosated N^G-hydroxy-agmatine, a nitrosylated N^G-hydroxy-agmatine, a nitrosated N^G-hydroxy-L-argininic acid, or a nitrosylated N^G-hydroxy-L-argininic acid.

53. The pharmaceutical composition of claim 52, wherein the analog of N-hydroxy-L-arginine is the nitrosated N-hydroxy-L-arginine or the nitrosylated N-hydroxy-L-arginine.

54. The pharmaceutical composition of claim 53, wherein the nitrosated N-hydroxy-L-arginine or the nitrosylated N-hydroxy-L-arginine is an adduct of N-hydroxy-L-arginine with nitric oxide.

55. The pharmaceutical composition of claim 49, wherein the N-

hydroxyguanidine compound is a substrate for nitric oxide synthase.

56. The pharmaceutical composition of claim 49, wherein the pharmaceutical composition is in a form that can be administered orally, parenterally, topically, vaginally, by inhalation, or by transurethral application.

57. The pharmaceutical composition of claim 49, further comprising at least one vasoactive agent.

58. The pharmaceutical composition of claim 57, wherein the vasoactive agent is an α -blocker, a calcium blocker, a β -blocker, a phosphodiesterase inhibitor, adenosine, an ergot alkaloid, a vasoactive intestinal peptide, a dopamine agonist, an opioid antagonist, a prostaglandin, an endothelin antagonist, a potassium channel activator or a mixture thereof.

59. The pharmaceutical composition of claim 58, wherein the vasoactive agent is an α -blocker.

60. The pharmaceutical composition of claim 58, wherein the vasoactive agent is a phosphodiesterase inhibitor.

61. The pharmaceutical composition of claim 58, wherein the vasoactive agent is a dopamine agonist.

62. The pharmaceutical composition of claim 58, wherein the vasoactive agent is a prostaglandin.

63. The pharmaceutical composition of claim 58, wherein the vasoactive agent is an endothelin antagonist.

64. The pharmaceutical composition of claim 58, wherein the vasoactive agent is a potassium channel activator.

65. A kit comprising a therapeutically effective amount of at least one N-hydroxyguanidine compound.

66. The kit of claim 65, wherein the N-hydroxyguanidine compound is N-aryl-N'-hydroxyguanidine, a nitrosated N-aryl-N'-hydroxyguanidine, a nitrosylated N-aryl-N'-hydroxyguanidine, N-hydroxy-L-arginine, or an analog of N-hydroxy-L-arginine.

67. The kit of claim 66, wherein the N-hydroxyguanidine compound is N-hydroxy-L-arginine.

68. The kit of claim 66, wherein the analog of N-hydroxy-L-arginine is a

N^ω-hydroxy-homo-L-arginine, a carboxylic ester of N-hydroxy-L-arginine, a N-α derivative of N-hydroxy-L-arginine, N^G-hydroxy-arginine, N^G-hydroxy-L-argininic acid, a nitrosated N-hydroxy-L-arginine, a nitrosylated N-hydroxy-L-arginine, a nitrosated N^ω-hydroxy-homo-L-arginine, a nitrosylated N^ω-hydroxy-homo-L-arginine, a nitrosated carboxylic ester of N-hydroxy-L-arginine, a nitrosylated carboxylic ester of N-hydroxy-L-arginine, a nitrosated N-α derivative of N-hydroxy-L-arginine, a nitrosylated N-α derivative of N-hydroxy-L-arginine, a nitrosated N^G-hydroxy-arginine, a nitrosylated N^G-hydroxy-arginine, a nitrosated N^G-hydroxy-L-argininic acid, or a nitrosylated N^G-hydroxy-L-argininic acid.

69. The kit of claim 68, wherein the analog of N-hydroxy-L-arginine is the nitrosated N-hydroxy-L-arginine or the nitrosylated N-hydroxy-L-arginine.

70. The kit of claim 69, wherein the nitrosated N-hydroxy-L-arginine or the nitrosylated N-hydroxy-L-arginine is an adduct of N-hydroxy-L-arginine with nitric oxide.

71. The kit of claim 65, wherein the N-hydroxyguanidine compound is a substrate for nitric oxide synthase.

72. The kit of claim 65, further comprising at least one vasoactive agent.

73. The kit of claim 72, wherein the vasoactive agent is an α-blocker, a calcium blocker, a β-blocker, a phosphodiesterase inhibitor, adenosine, an ergot alkaloid, a vasoactive intestinal peptide, a dopamine agonist, an opioid antagonist, a prostaglandin, an endothelin antagonist, a potassium channel activator or a mixture thereof.

1/21

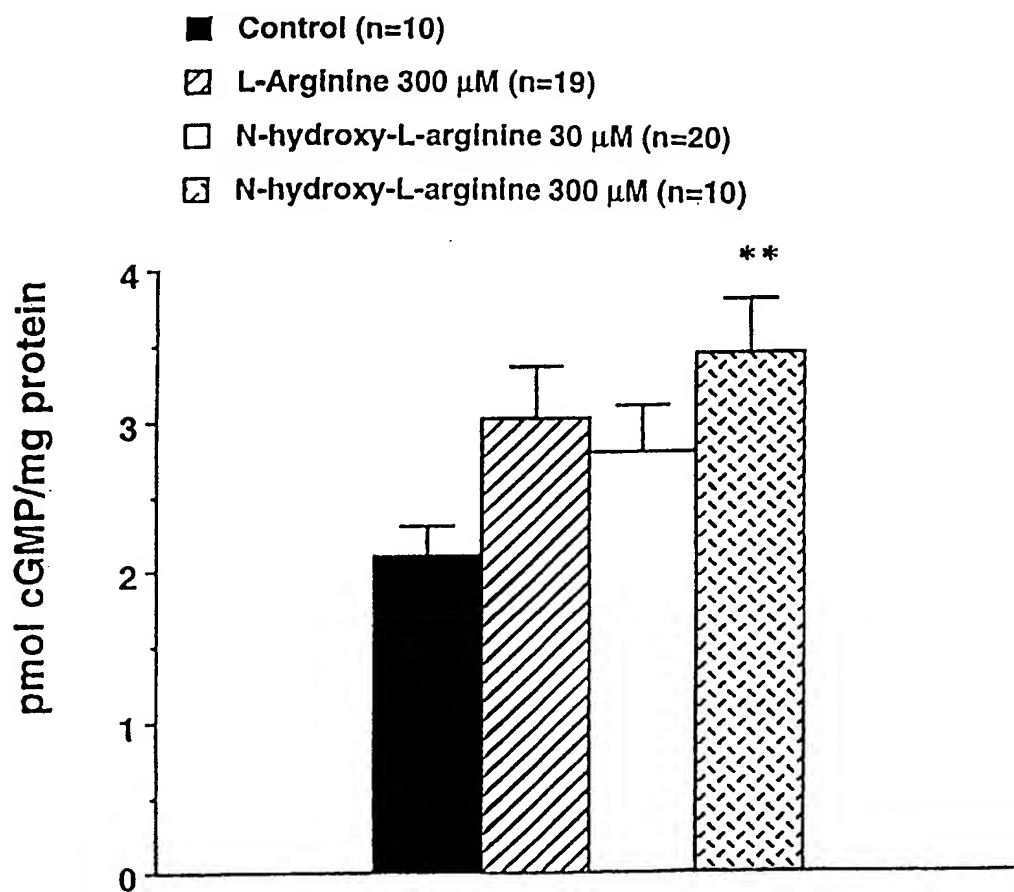


FIG. 1

BEST AVAILABLE COPY

2/21

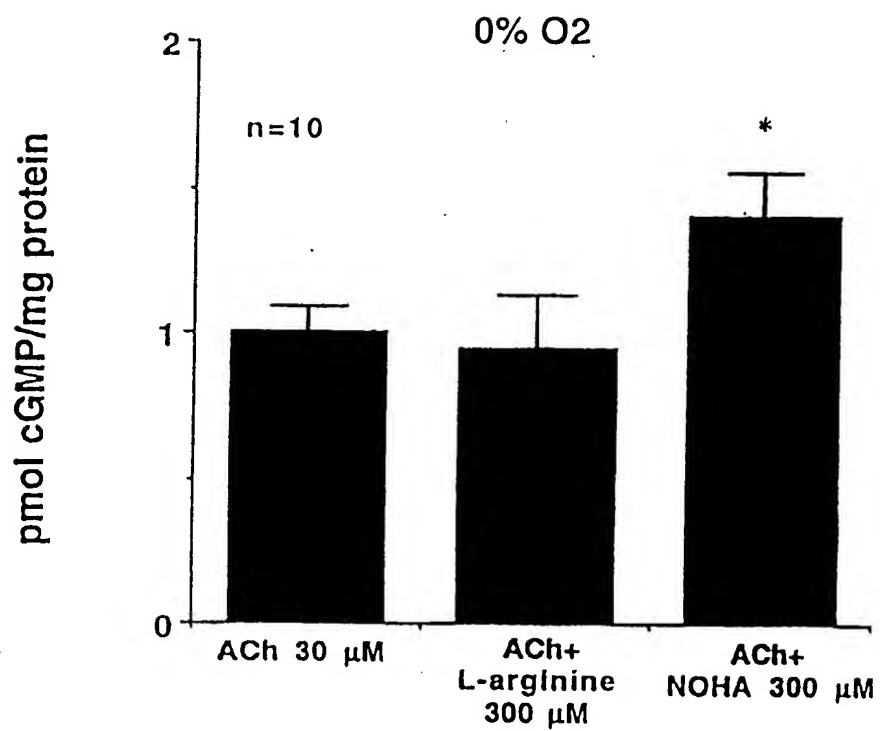


FIG.2A

3/21

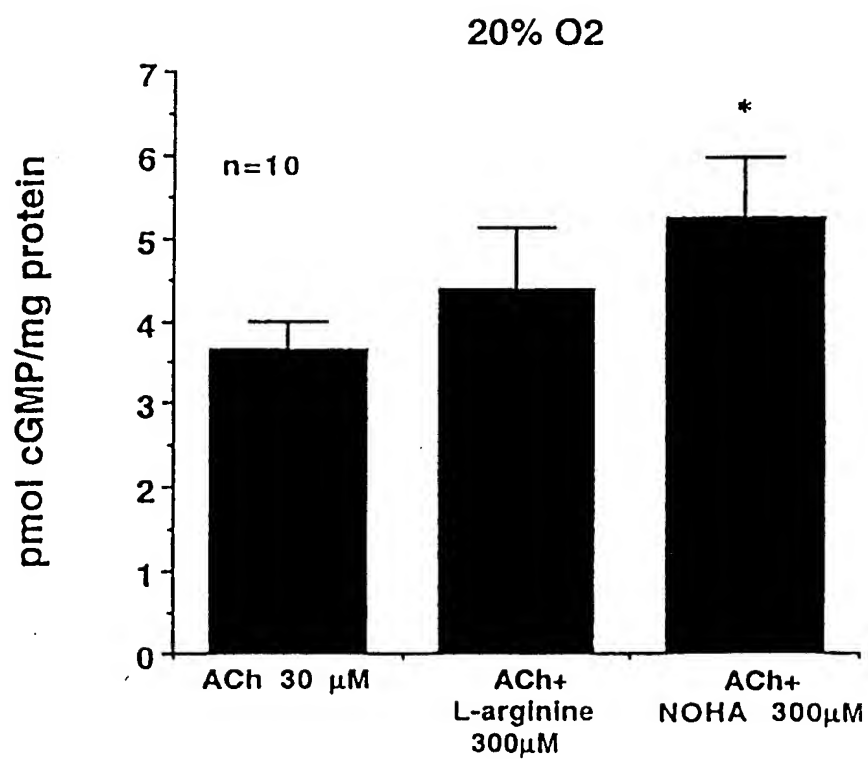


FIG.2B

4/21

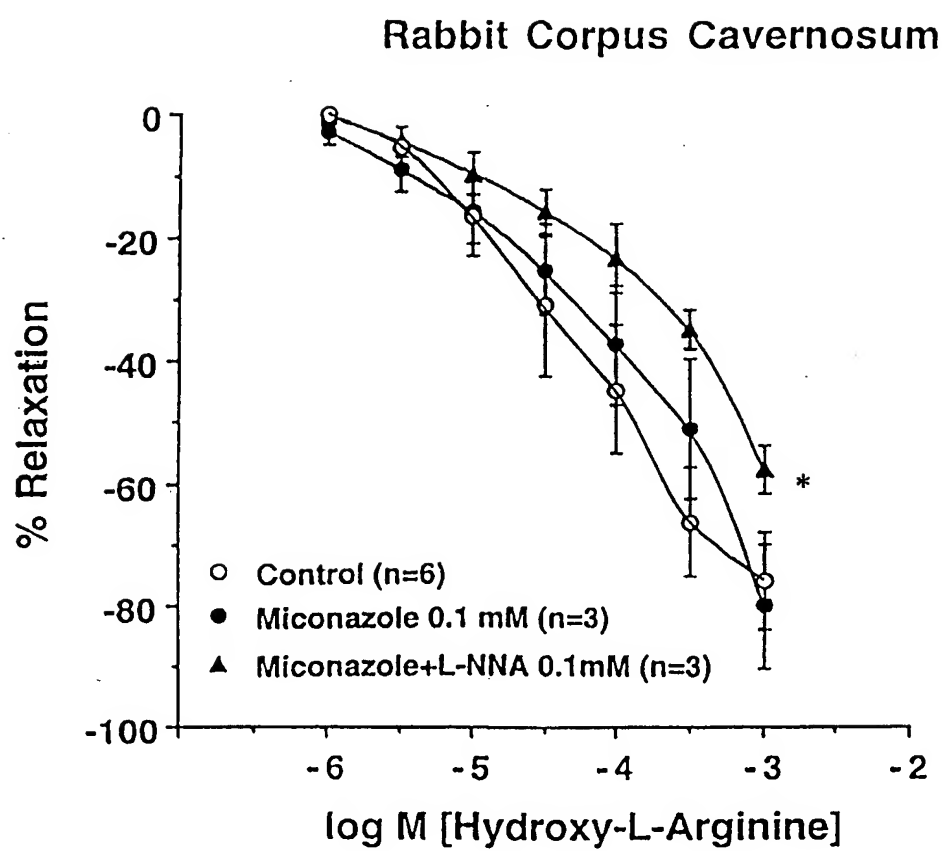


FIG.3

5/21

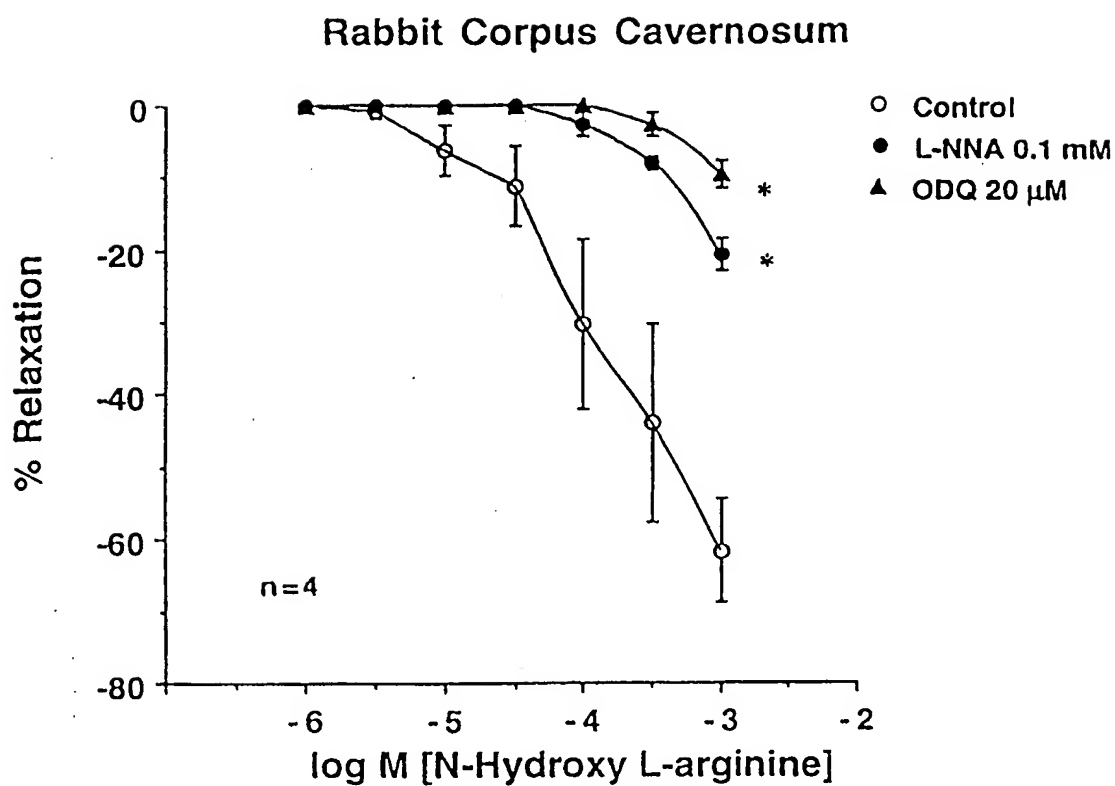


FIG.4A

6/21

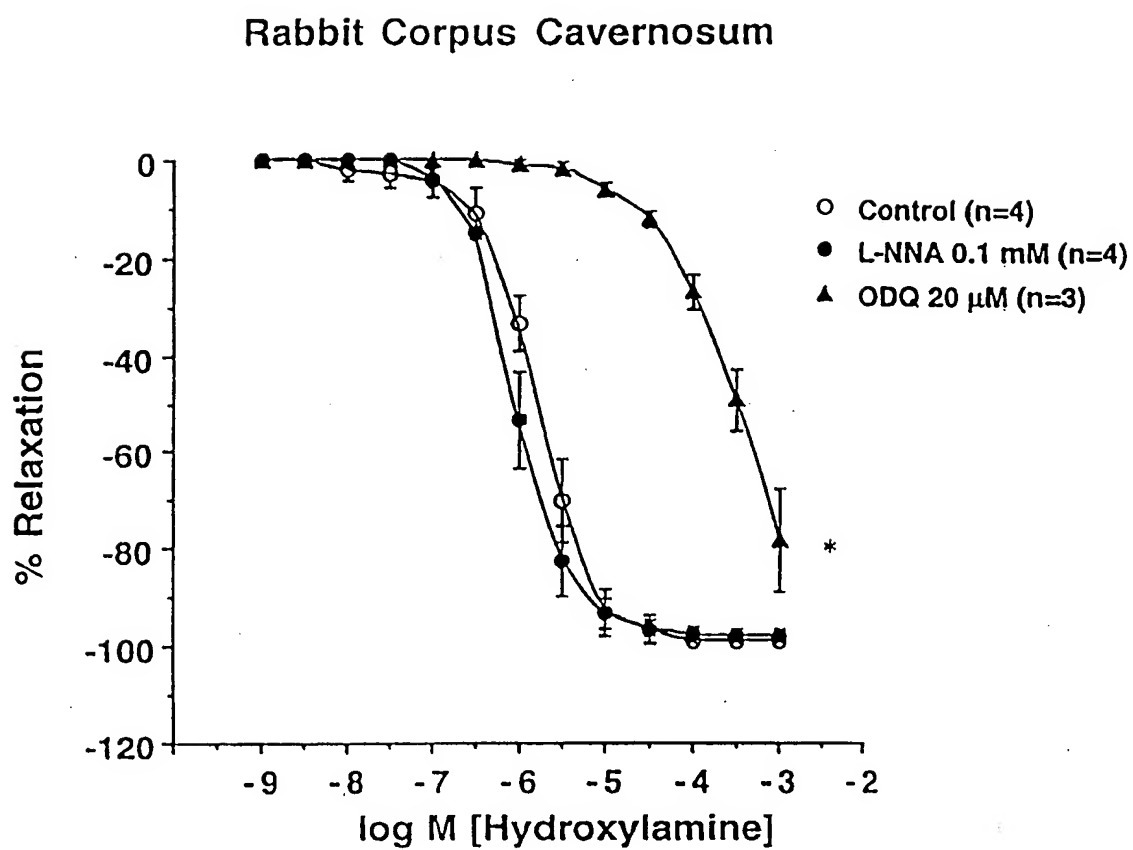


FIG.4B

7/21

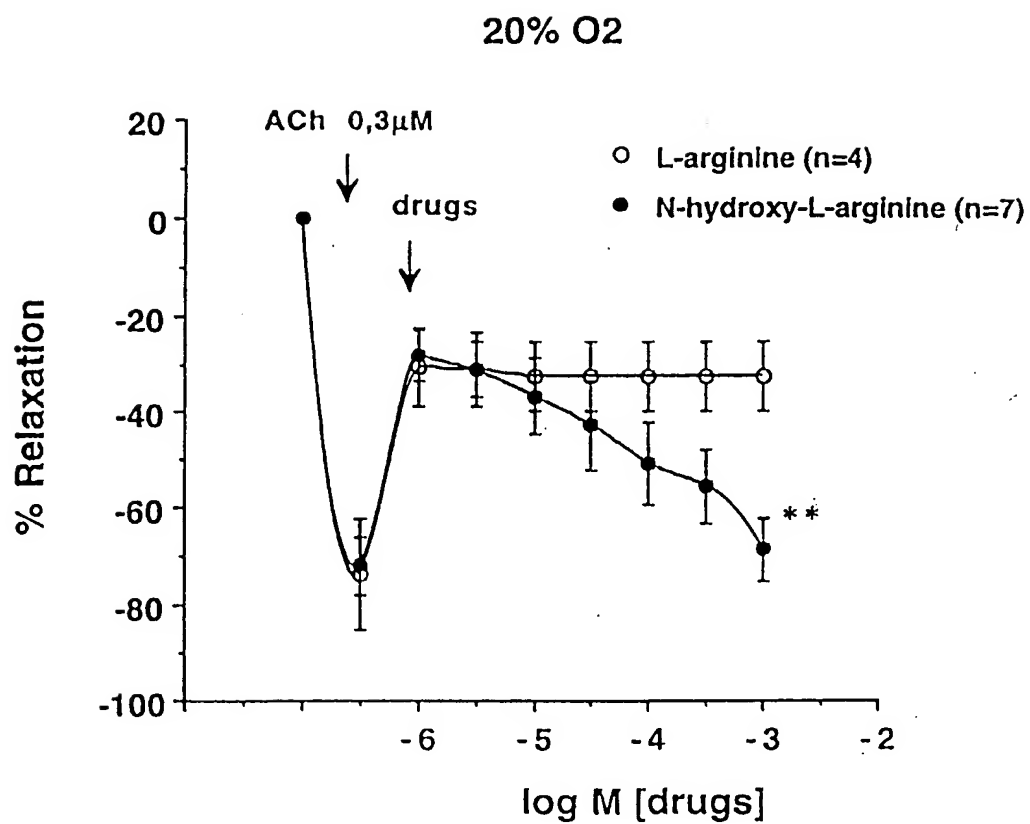


FIG.5A

8/21

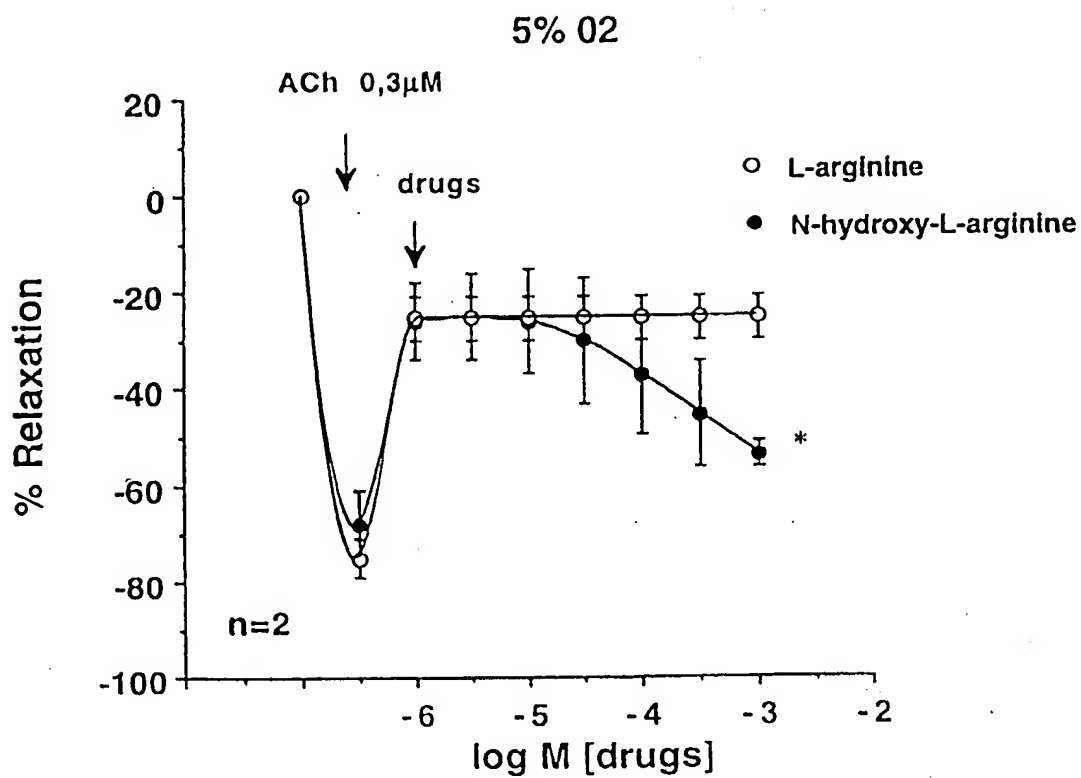


FIG.5B

9/21

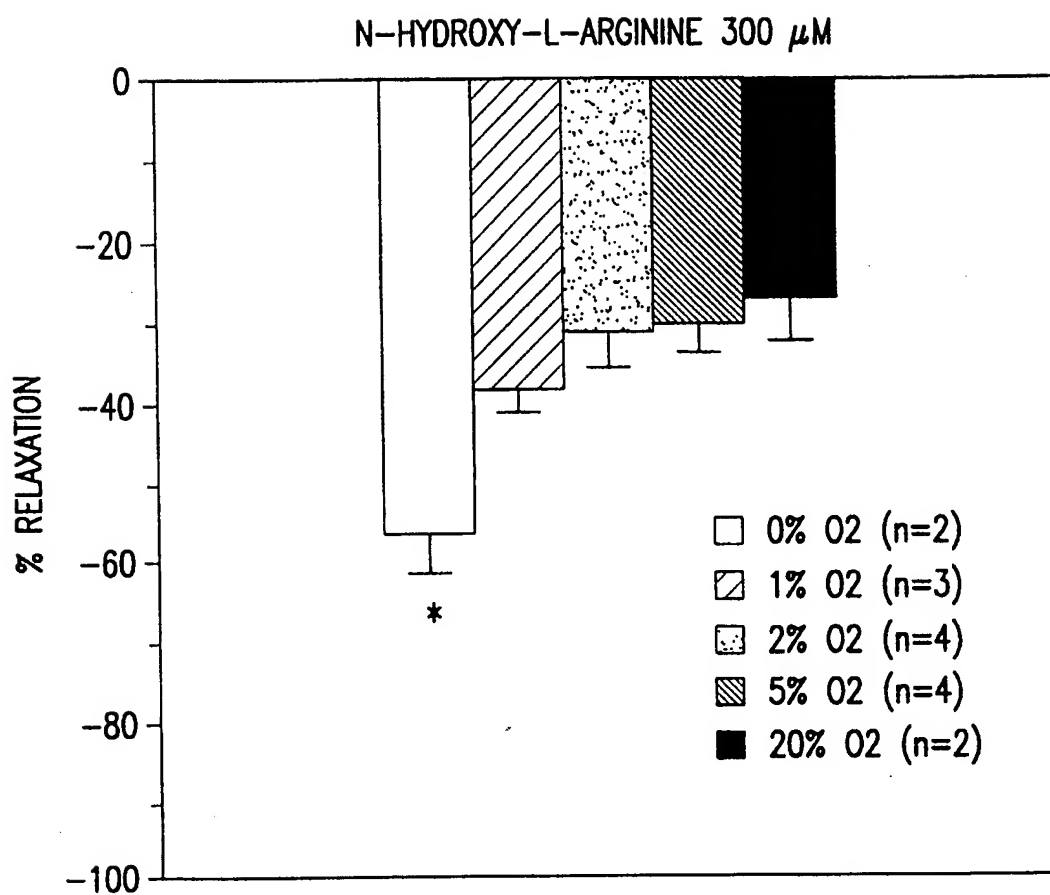


FIG.6

10/21

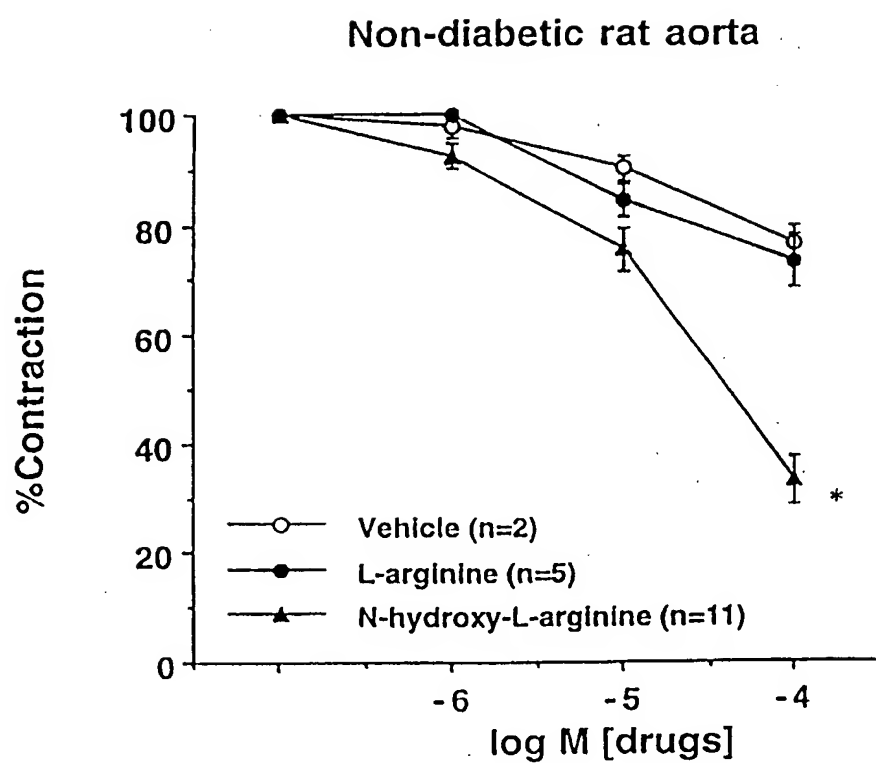
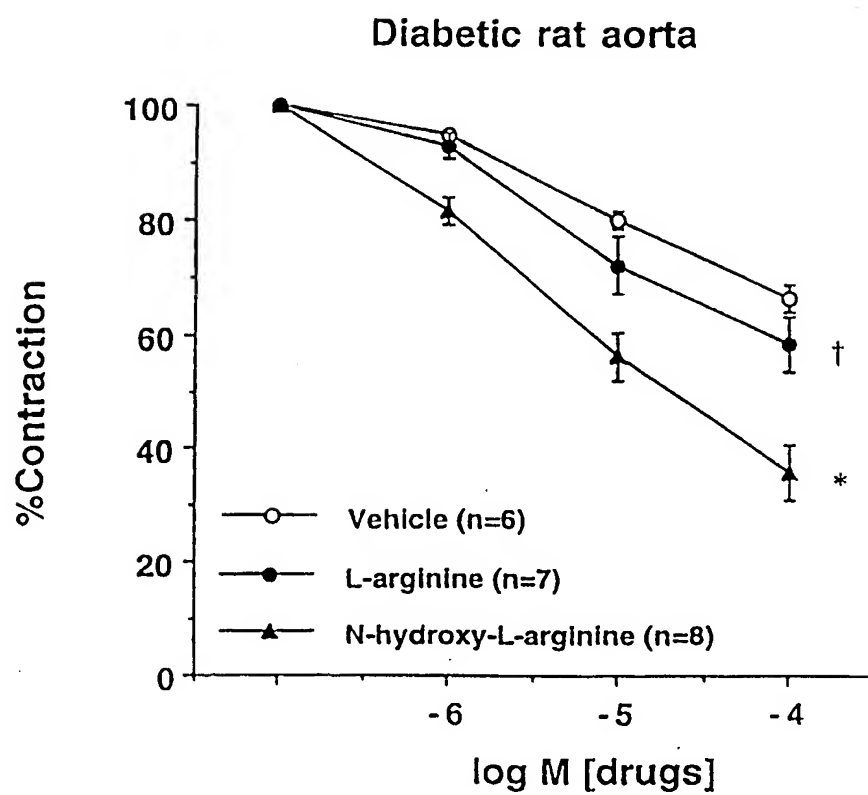


FIG.7A

11/21

**FIG.7B**

12/21

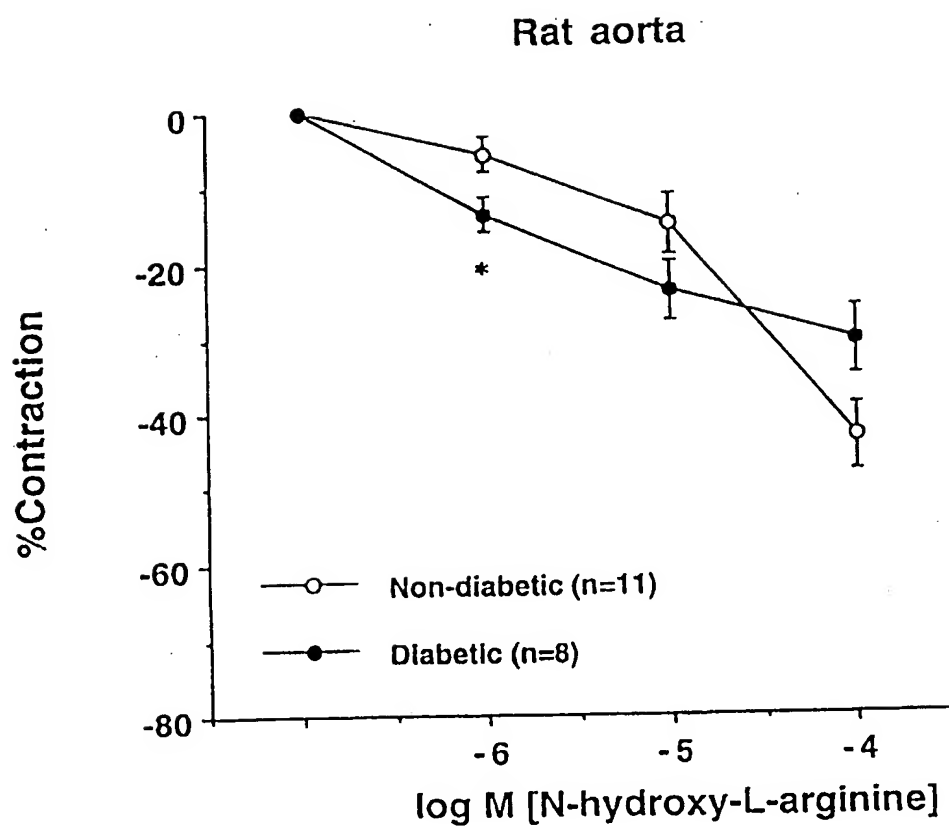


FIG.8

13/21

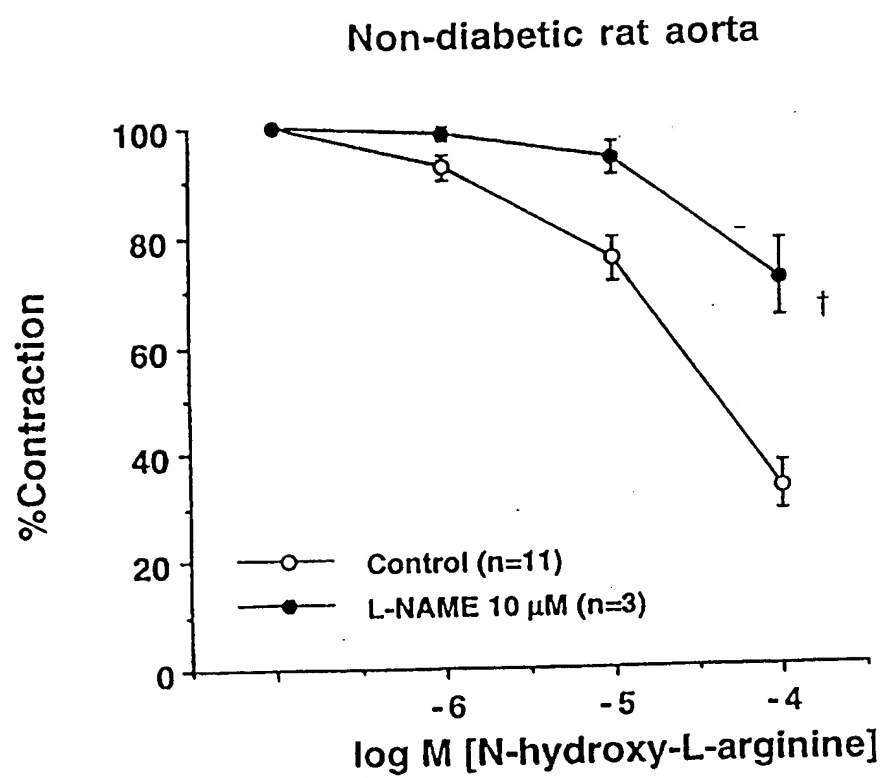


FIG.9A

14/21

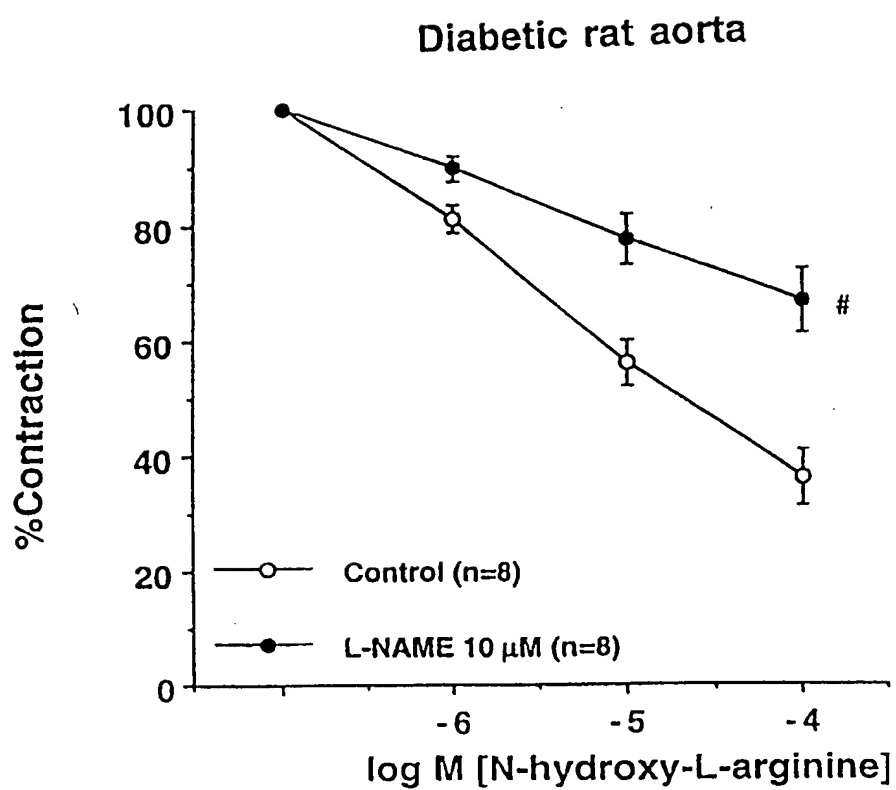


FIG.9B

15/21

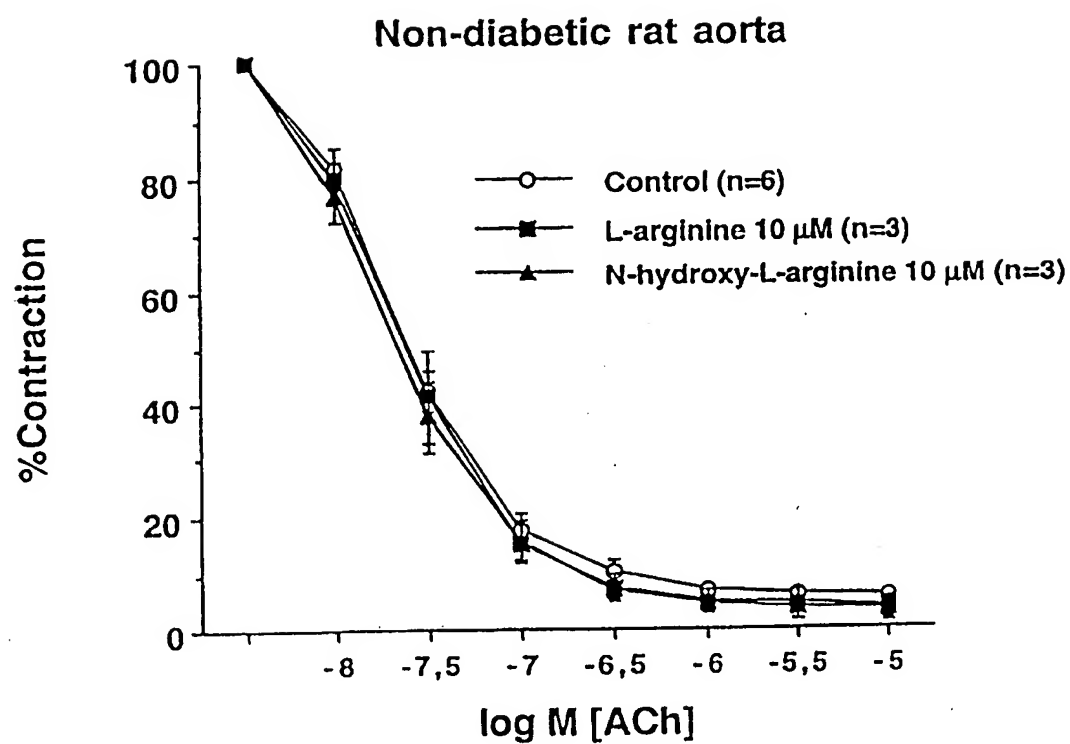


FIG.10A

16/21

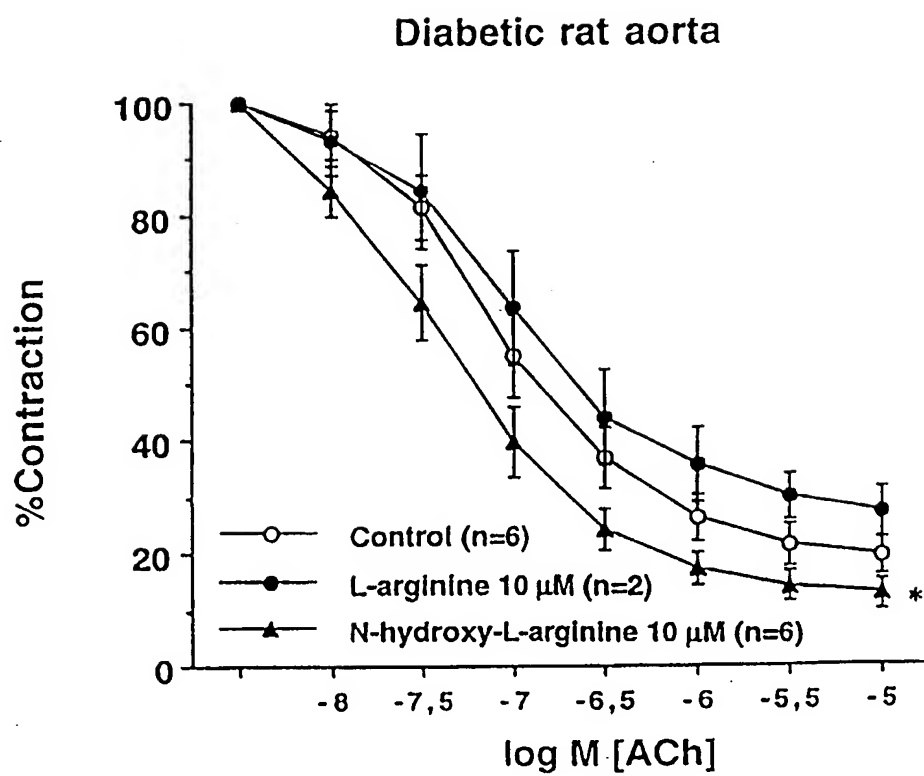


FIG.10B

17/21

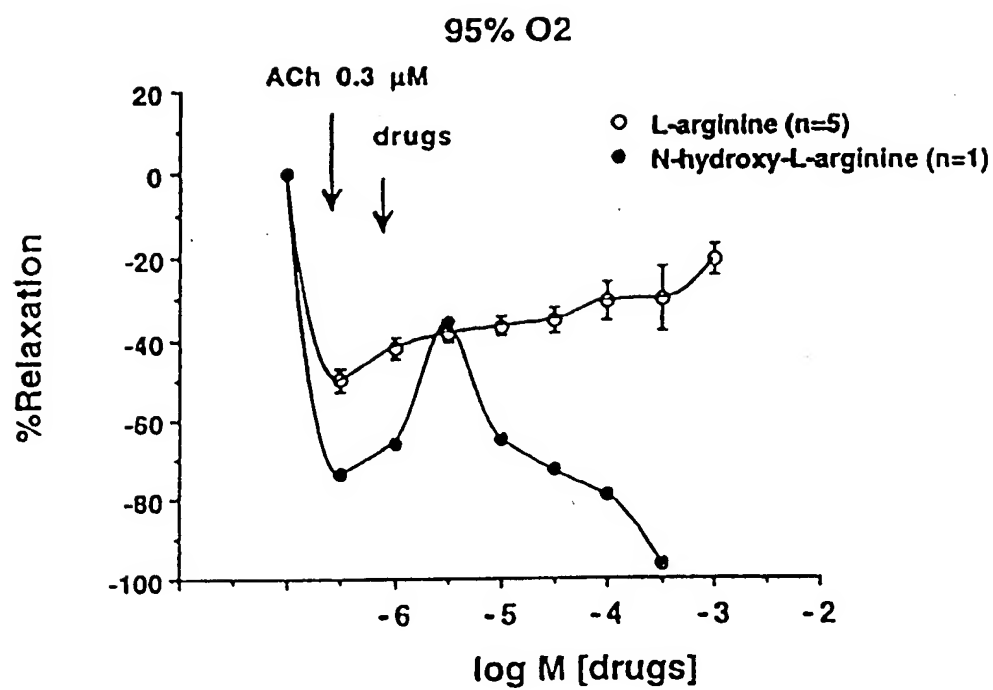


FIG.11A

18/21

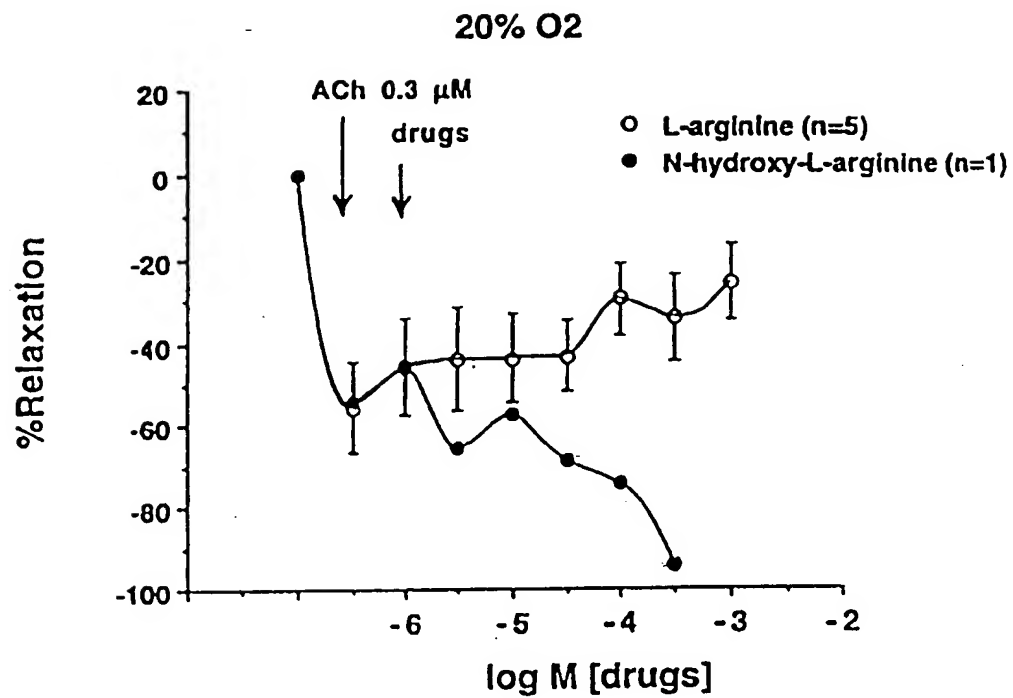


FIG.11B

19/21

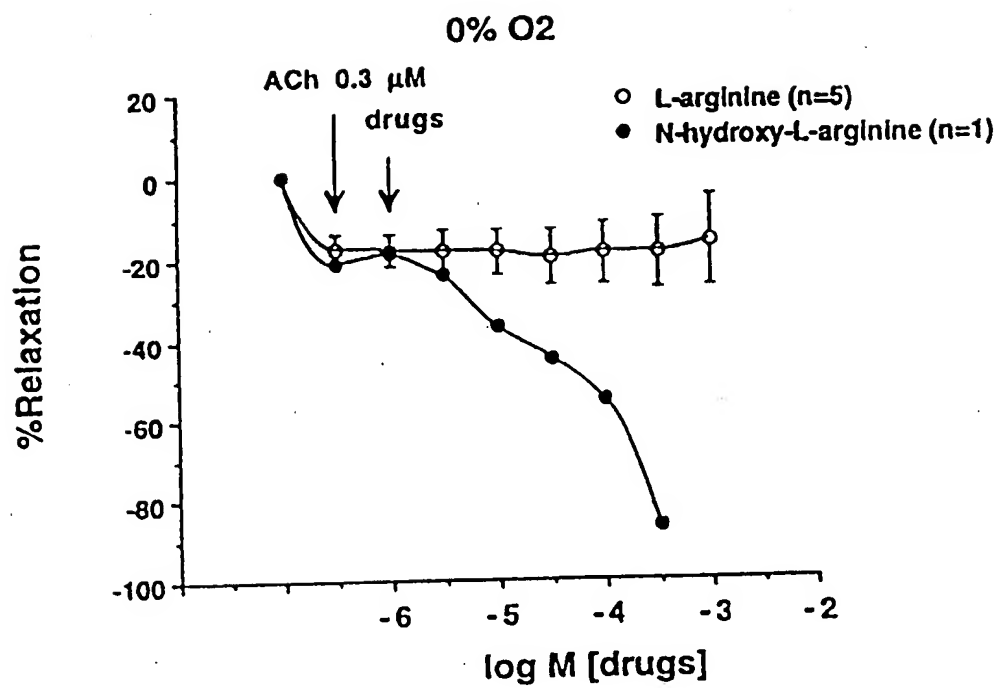


FIG.11C

20/21

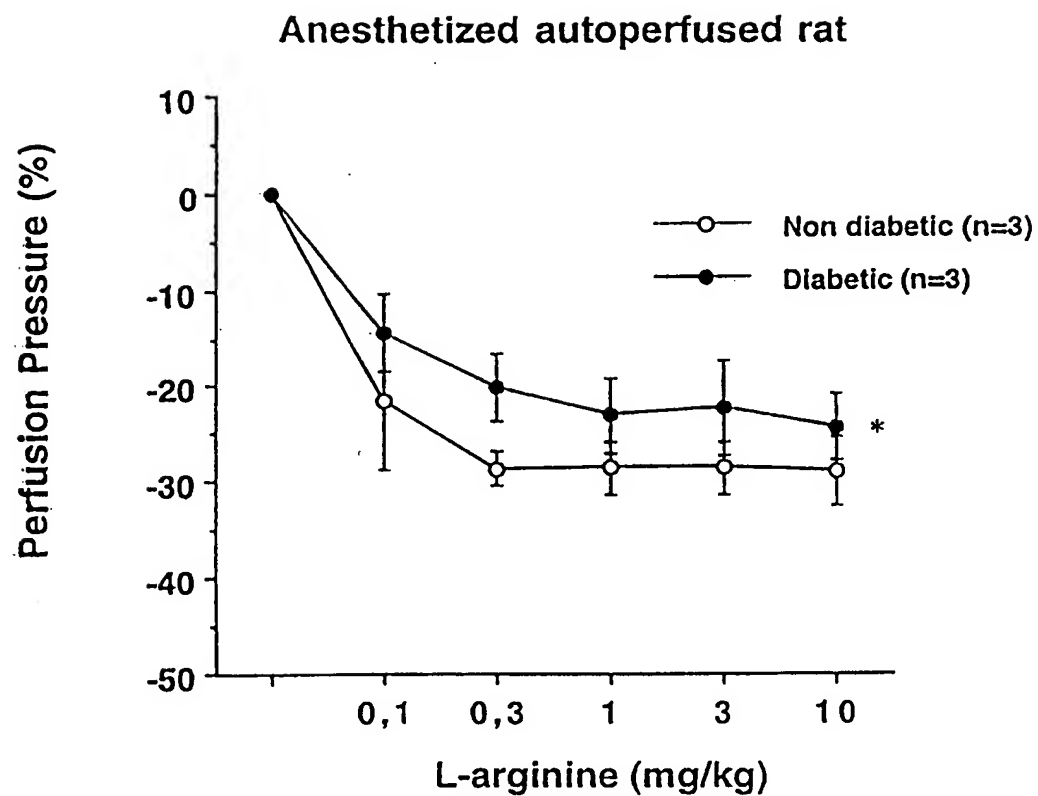


FIG.12A

21/21

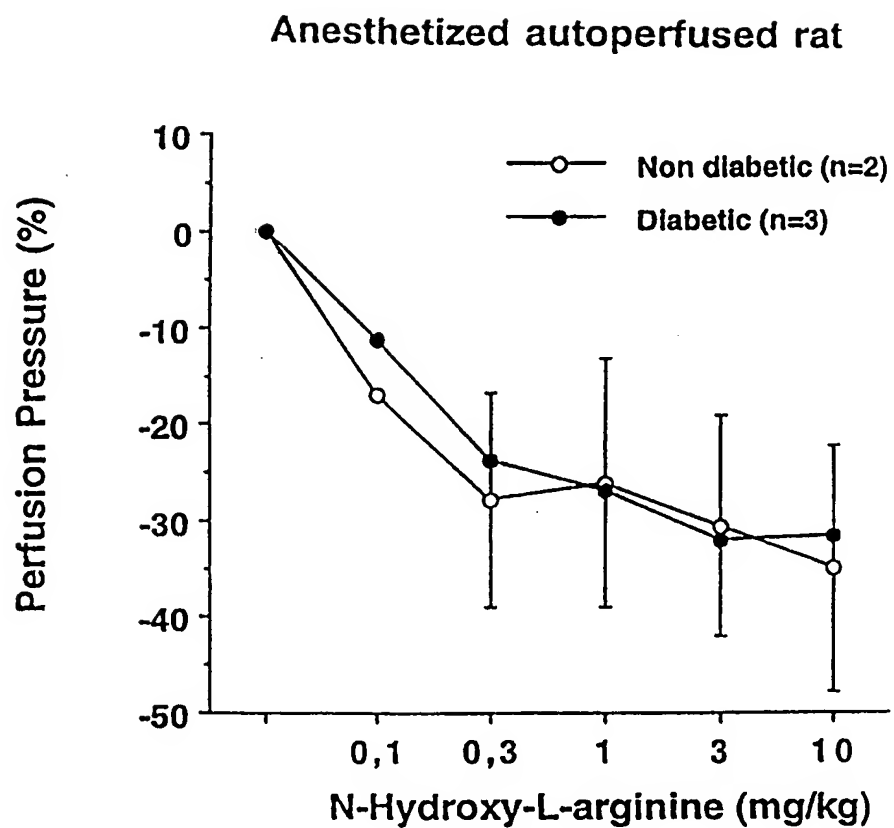


FIG.12B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11876

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 31/195
US CL : Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/564

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, STN:
N-hydroxy-L-arginine, erectile, erection, penis.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	US 5,906,987 A (CHWALISZ et al.) 25 May 1999, see entire documnet.	1-73

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
B earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
20 JULY 1999

Date of mailing of the international search report
19 OCT 1999

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
THEODORE J. CRIARES
Telephone No. (703) 308-1235

JOYCE BRIDGERS
PARALEGAL SPECIALIST
CHEMICAL MATRONS

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/11876

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

514/564

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 31/195, 31/21, 31/295, 31/34 // (A61K 31/195, 31:56, 31:57, 31:565, 31:00) (A61K 31/295, 31:56, 31:57, 31:565, 31:00) (A61K 31/21, 31:56, 31:57, 31:565, 31:00) (A61K 31/34, 31:56, 31:57, 31:565, 31:00)	A1	(11) International Publication Number: WO 97/25984 (43) International Publication Date: 24 July 1997 (24.07.97)
(21) International Application Number: PCT/US97/00795 (22) International Filing Date: 21 January 1997 (21.01.97) (30) Priority Data: 08/588,586 18 January 1996 (18.01.96) US (71) Applicants: SCHERING AKTIENGESELLSCHAFT [DE/DE]; Muellerstrasse 178, D-13342 Berlin (DE). BOARD OF REGENTS THE UNIVERSITY OF TEXAS SYSTEM [US/US]; 201 West Seventh Street, Austin, TX 78701 (US). (72) Inventors: GARFIELD, Robert, E.; 1814 Winding Way, Friendswood, TX 77546 (US). CHWALISZ, Kristof; Lobbersteig 70, D-13503 Berlin (DE). (74) Agents: HAMLET-KING, Diana et al.; Millen, White, Zelano & Branigan, P.C., Arlington Courthouse Plaza I, Suite 1400, 2200 Clarendon Boulevard, Arlington, VA 22201 (US).	(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(54) Title: TREATMENT OF URINARY INCONTINENCE WITH NITRIC OXIDE SYNTHASE SUBSTRATES AND/OR NITRIC OXIDE DONORS		
(57) Abstract The invention provides a method for the treatment and prevention of urinary incontinence in mammals, e.g., human males and females, especially nonpregnant female mammals, by administering a nitric oxide synthase substrate and/or nitric oxide donor, alone or in combination with an estrogenic agent and/or a progestational substance, with or without supplementation with an alpha-adrenergic agonist, beta-adrenergic receptor blocking agent, cholinergic-receptor blocking compound or a cholinergic-receptor-stimulating drug, as well as pharmaceutical compositions useful in practicing the methods of this invention.		